New Triterpenes from *Gymnema sylvestre*

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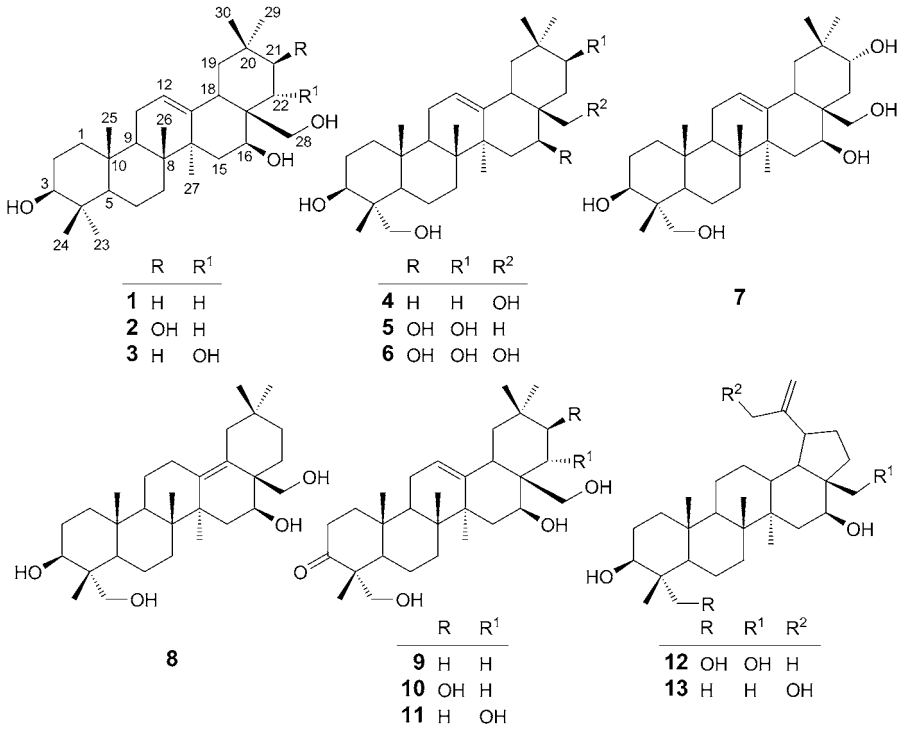
Phytochemical investigation of the aerial parts of *Gymnema sylvestre* has led to the isolation of seven new triterpenes, six oleane types (**5**, **7**–**11**) and a new lupane type (**12**), and of the six known analogues **1**–**4**, **6**, and **13**. The structures and relative configurations of these compounds were elucidated by spectroscopic analyses, including 1D- and 2D-NMR spectroscopy and mass spectrometry, and by the comparison of their NMR data with those of related compounds.

Introduction. – *Gymnema sylvestre* is a bushy climber of the Asclepiadaceae family that is found mainly in the Central and Tropical Indian Peninsula, Sri Lanka, and Africa. It is considered a medicinal plant and is used in folk medicine, ayurveda, and homeopathy for treatment of asthma, eye disorders, inflammation, and snake bites, as a tonic and refrigerant, diuretic, laxative, sedative for coughs, and especially as an antidote for diabetes [1]. Recent studies, based on the historical evidence of the many uses in the medicinal field, have indicated that the leaves and roots of the plant are the parts that contain the biologically active ingredients [2]. The active compounds of *G. sylvestre* are called gymnemic acids [3]. Their use in the past to counteract the effects of excess glucose in diabetes mellitus explains the Hindi name of the plant, '*Gur-ma*' which means 'destroyer of sugar'. The plant extracts have been reported to exert an antimicrobial and hepatoprotective activity, and to lower the levels of triglycerides and LDL cholesterol, and raise the HDL cholesterol ('good cholesterol') [4][5]. They also act as a deterrent against *Prodenia eridania*, prevent dental caries caused by *Streptococcus mutans*, and are used in skin cosmetics [6–8].

In an investigation of *G. sylvestre*, we have isolated five known oleanane-type triterpenes, **1**–**4** and **6**, six new analogs, **5** and **7**–**11**, and two lupane-type triterpenes, **12** and **13**, the former of which was new.

Results and Discussion. – The CH_2Cl_2 extract of the aerial parts of *G. sylvestre* was separated into acidic and neutral fractions with 2N NaOH. The neutral fraction, washed with H_2O and concentrated under reduced pressure, was filtered through SiO_2 with petroleum ether, CH_2Cl_2, AcOEt, Me_2CO, MeOH, and H_2O, as eluents of increasing polarity. Column chromatography of the fraction eluted with CH_2Cl_2 yielded



compounds **1**–**13**. The known triterpenes **1**–**4**, **6**, and **13** were identified as 3*β*,16*β*,28-trihydroxyolean-12-ene (**1**) [9], 3*β*,16*β*,21*β*,28-tetrahydroxyolean-12-ene (**2**) [10], 3*β*,16*β*,22*α*,28-tetrahydroxyolean-12-ene (**3**) [11], 3*β*,23,28-trihydroxyolean-12-ene (**4**) [12], 3*β*,16*β*,21*β*,23,28-pentahydroxyolean-12-ene (**6**) [13], and 3*β*,16*β*,29-trihydroxylup-20(30)-ene (**13**) [14].

The new compounds were characterized by various spectroscopic methods. The ESI-MS of compound **5** showed a *quasi*-molecular-ion peak at *m*/*z* 475.2 ($[M+H]^+$), suggesting the molecular formula $C_{30}H_{50}O_4$ and six degrees of unsaturation. Fragment ions at *m*/*z* 457.5 ($[M-H_2O+H]^+$), 439.3 ($[M-2\,H_2O+H]^+$), and *retro-Diels–Alder* fragmentation ion, characteristic of an oleanane skeleton, at *m*/*z* 250.4 (50%) evidenced an olean-12-ene derivative. The ^{1}H- and ^{13}C-NMR spectra of **5** confirmed this assumption. The ^{1}H-NMR spectrum showed signals of five H-atoms geminal to an O-bearing function as three *double doublet*s at δ(H) 4.10, 3.61, 3.52, a *doublet* at 3.53, and a signal at δ(H) 3.31, partly obscured by solvent. The ^{13}C-NMR spectrum (*Table*) exhibited signals of three O-bearing CH groups at δ(C) 74.5, 74.3, 67.8 and of a O-bearing CH_2 group at δ(C) 67.8. In the upfield region of ^{1}H-NMR, seven Me *singlet*s were detected at δ(H) 1.23, 1.02, 1.01, 0.95, 0.87, 0.81, and 0.71. The analysis of COSY, NOESY, HSQC, and HMBC spectra allowed us to determine the structure of 3*β*,16*β*,21*β*,23-tetrahydroxyolean-12-ene for this compound. The ^{1}H,^{1}H-COSY experiment showed correlations between H–C(16) (δ(H) 4.10) and CH_2(15) (δ(H) 1.67 and 1.25); H–C(21) (δ(H) 3.52) and CH_2(22) (δ(H) 1.68 and 1.60); and H–C(3) (δ(H)

Table. *^{13}C-NMR Data of Triterpenes* **5**, **7**–**12** (at 125 MHz, in CD_3OD; δ in ppm)

C-Atom	**5**	**7**	**8**	**9**	**10**	**11**	**12**
C(1)	40.1	40.1	40.2	39.4	39.1	39.3	40.2
C(2)	25.1	27.9	28.1	37.1	37.5	37.1	28.1
C(3)	74.3	74.3	74.4	219.2	220.1	218.8	74.2
C(4)	43.8	43.7	42.6	51.6	53.9	54.0	43.9
C(5)	48.5	49.3	49.0 (obs)[a])	48.7	47.2	47.3	49.4
C(6)	19.6	19.6	19.7	21.0	20.9	21.0	19.5
C(7)	33.9	34.0	36.3	33.5	33.4	33.4	35.4
C(8)	43.8	41.3	43.9	38.0	41.4	41.8	43.9
C(9)	49.0	48.7	52.1	47.4	48.6	48.5	51.9
C(10)	37.7	37.6	38.7	40.0	41.4	37.7	38.6
C(11)	25.1	25.1	23.9	25.3	25.2	25.3	22.4
C(12)	124.5	124.7	27.0	124.4	124.8	125.6	26.7
C(13)	142.3	144.5	139.9	145.2	143.7	142.2	38.5
C(14)	45.2	45.3	48.0	42.2	45.2	44.2	46.2
C(15)	37.0	36.6	37.3	37.1	36.9	36.5	39.0
C(16)	67.8	71.7	78.2	68.3	68.9	69.8	80.0
C(17)	40.1	45.3	45.1	41.8	45.2	47.5	46.2
C(18)	50.5	44.6	129.8	45.6	44.6	43.3	49.8
C(19)	41.6	43.8	40.3	48.1	48.6	47.8	49.6
C(20)	38.2	36.5	34.2	32.2	37.7	37.8	151.6
C(21)	74.5	76.2	36.0	35.3	74.4	78.7	31.5
C(22)	28.8	33.1	30.7	26.6	34.2	74.4	33.9
C(23)	67.8	67.8	67.9	68.5	68.3	69.4	67.7
C(24)	13.2	13.2	13.1	18.5	18.3	18.5	13.0
C(25)	17.0	17.0	17.8	16.4	16.3	16.3	17.5
C(26)	18.1	18.0	19.0	17.8	17.7	17.8	17.7
C(27)	28.1	27.3	23.2	27.9	27.7	28.1	16.8
C(28)	22.9	68.1	64.5	69.5	68.4	59.6	62.4
C(29)	30.3	28.2	32.9	31.8	30.1	30.6	111.1
C(30)	18.3	26.0	25.8	24.8	18.0	19.2	19.9

[a]) obs: Obscured by the solvent.

3.61) and CH_2(2) (δ(H) 1.96 and 1.93). The latter correlated with the CH_2(1) (δ(H) 1.65, 1.16). Finally, the signal of the olefinic H-atom H–C(12) at δ(H) 5.28 correlated with H–C(11) (δ(H) 1.96 and 1.93). The correlations in the HMBC experiment between the H-atom signal at δ(H) 4.10 and that of the H-atoms of Me(28) at δ(H) 0.81 with the CH signal at δ(C) 50.5, assigned to C(18) and bonded to H-atom with the signal at δ(H) 2.20, and between the signal of H–C(18) with that of the O-bearing H–C(16) at δ(C) 67.8, indicated an OH group at C(16). The correlations between the signals of H–C(23) at δ(H) 3.53 and 3.31 with that of the CH at δ(C) 48.5, assigned to C(5) and bonded to the H-atom with the signal at δ(H) 1.62, and between this signal and that of the O-bearing CH_2 at δ(H) 67.8 localized another OH group at C(23). Finally, the correlations of the signals of two geminal Me groups, *i.e.*, Me(29) at δ(H) 0.95 and Me(30) at δ(H) 0.87, with the C-atom signal at δ(C) 74.5 defined a further hydroxylation site at C(21). These data, compared with those of the other isolated triterpenes, were in accordance with a 3,16,21,23-tetrahydroxyoleanane skeleton. The

configurations of the OH groups were deduced from a NOESY experiment (*Fig.*), where H–C(16) showed NOE effects with the H-atoms of Me(27) and H-atom H–C(19) (δ(H) 2.10), indicating a β-orientation for the OH group at C(16). The H–C(21) showed NOE effects with the H-atoms of Me(29), H–C(19), and H–C(16) indicating also a β-orientation for the OH group at C(21).

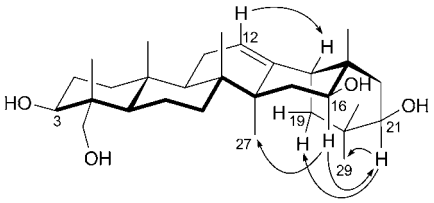


Figure. *Key NOE correlations for compound* **5**

The ESI-MS of compound **7** showed a *quasi*-molecular-ion peak at *m*/*z* 491.4 ($[M+H]^+$), suggesting the molecular formula $C_{30}H_{50}O_5$ and six degrees of unsaturation. Fragment-ion peaks at *m*/*z* 473.4 ($[M-H_2O+H]^+$), 455.3 ($[M-2\,H_2O+H]^+$), and *retro-Diels–Alder* fragmentation-ion peak, characteristic of an oleanane skeleton, at *m*/*z* 267.5 (30%) evidenced an olean-12-ene derivative. The ^{1}H- and ^{13}C-NMR data were similar to those of triterpene **6**, whose structure had been previously reported in [13]. A concise analysis of its spectroscopic data allowed us to determine the structure of **7** as the *C(21)*-epimer of the reference compound **6**. The ^{1}H,^{1}H-COSY experiment showed correlations between: the *double doublet* H–C(16) at δ(H) 4.57 and the CH_2(15) at δ(H) 1.80 and 1.30, the broad *triplet* H–C(21) at δ(H) 3.52 and the CH_2(22) at δ(H) 2.14 and 1.77, abd the *double doublet* H–C(3) at δ(H) 3.62 and the CH_2(2) at δ(H) 1.71. The correlations in the HMBC experiment between the two geminal Me(29) and Me(30) at δ(H) 0.92 and 0.95, respectively, with C-atom at δ(C) 76.2 indicated a hydroxylation site at C(21). The configuration at this C-atom has been defined by a NOE experiment where H–C(21) showed NOE effects with both Me(29) and Me(30), indicating an α-orientation of the OH group. This assumption was supported by the multiplicity of H–C(21) signal, which appeared as a broad *triplet* at 3.52 ($J=$ 3.0).

The ESI-MS of compound **8** displayed a *quasi*-molecular-ion peak at *m*/*z* 475.5 ($[M+H]^+$), indicating the molecular formula $C_{30}H_{50}O_4$ with six degrees of unsaturation. Fragment-ion peaks at *m*/*z* 457.3 ($[M-H_2O+H]^+$), 439.2 ($[M-2\,H_2O+H]^+$) were also detected. The ^{1}H-NMR spectrum showed signals of six geminal H-atoms bound to an oxygenated function (two as *double doublet*s at δ(H) 3.72 and 3.62, and three as *doublet*s at δ(H) 3.82, 3.76, and 3.54, and one at δ(H) 3.30, obscured by the solvent). In the upfield region, six Me *singlet*s were evident at δ(H) 1.26, 0.96, 2 × 0.92, 0.76, and 0.68. The ^{13}C-NMR (*Table*) showed two O-bearing CH signals at δ(C) 78.2 and 74.4, and those of two O-bearing CH_2 groups at δ(C) 67.9 and 64.5. The presence of two sp^2 quaternary C-atoms (δ(C) 139.9 and 129.8) indicated a C(13)=C(18) bond. Analysis of COSY, NOESY, HSQC, and HMBC spectra allowed us to determine the structure of 3β,16β,23,28-tetrahydroxyolean-13,18-ene for this compound. This proposal was confirmed by the HMBC of Me(27) with the downfield olefinic C(13) and correlations of H–C(16) and CH_2(28) with the upfield olefinic C(18). The ^{1}H,^{1}H-

COSY experiment showed correlations between H–C(16) (δ(H) 3.72) and CH_2(15) (δ(H) 1.98 and 1.35); and H–C(3) (δ(H) 3.62) and CH_2(2) (δ(H) 1.63). The latter correlated with CH_2(1) (δ(H) 1.71 and 1.05). Finally, H-atoms CH_2(12) (δ(H) 2.72 and 1.89) correlated with CH_2(11) (δ(H) 1.27) that correlated with H–C(9) (δ(H) 1.59). The correlations in the HMBC experiment between CH_2(23) (δ(H) 3.54 and 3.30) with the C-atom with a signal at δ(C) 49.0, assigned to C(5) and bonded to H-atom with the signal at δ(H) 1.18, and between this H-atom with the C-atom with the signal at δ(C) 74.4 evidenced a OH group at the C(23) position. In the NOESY experiment, the H–C(16) showed NOE effects with Me(27) and H-atom H_α–C(22), strongly suggesting a β-orientation for the OH group; moreover, one of the H-atoms Me(28) (δ(H) 3.82) showed a NOE with Me(26) (δ(H) 0.92), H–C(12) (δ(H) 2.72) showed a NOE effect with CH_2(19) (δ(H) 2.35), and this latter gave NOEs with Me(29) and Me(30) (δ(H) 0.96 and 0.76, resp.). The structure of this triterpene was reported some time ago by *Shibata et al.* [15] as a hydrogenation product of saikogenin A.

The ESI-MS of compound **9** showed a *quasi*-molecular-ion peak at *m*/*z* 473.2 ($[M+H]^+$), suggesting the molecular formula $C_{30}H_{48}O_4$ and seven degrees of unsaturation. Fragment-ion peaks at *m*/*z* 455.2 ($[M-H_2O+H]^+$), as well as an absorption band at 3376 cm^{-1} in the IR spectrum indicated the presence of OH groups. The *retro-Diels–Alder* fragmentation-ion peak, characteristic of the oleane skeleton, in particular the positively charged *D*/*E* ring-fragment peak at *m*/*z* 249.1 (42%) and the less abundant, positively charged *A*/*B* ring-fragment peak at *m*/*z* 225.4 (6%) evidenced an olean-12-ene derivative. The IR spectrum also showed absorption bands at 1118, 1090, and 1045 cm^{-1}, consistent with the presence of HO–C groups, and a band at 1722 cm^{-1} for the presence of a CO group. The ^{13}C-NMR (*Table*) spectrum showed 30 signals, which with the DEPT experiment, revealed the presence of six Me groups (δ(C) 31.8, 27.9, 24.8, 18.5, 17.8, and 16.4), an olefinic C-atom (δ(C) 124.4), an O-bearing CH group (δ(C) 68.3), and two O-bearing CH_2 groups (δ(C) 69.5 and 68.5). The ^{1}H-NMR spectrum indicated six tertiary Me groups (δ(H) 1.28, 1.11, 1.07, 0.98, and 2×0.93), an O-bearing CH group as *double doublet* (δ(H) 4.28), two O-bearing CH_2 groups (two *doublet*s at δ(H) 3.87 and 3.63), and it exhibited two signals (δ(H) 3.29 and 3.38), obscured by the solvent, and a trisubstituted olefinic H-atom signal at δ(H) 5.29 attributed to H–C(12). Analysis of COSY, NOESY, HSQC, and HMBC spectra allowed us to deduce the structure of 16β,23,28-trihydroxyolean-12-en-3-one for this compound. The ^{1}H,^{1}H-COSY experiment showed correlations between: H–C(16) (δ(H) 4.28) and CH_2(15) (δ(H) 1.88 and 1.43); CH_2(2) (δ(H) 2.48 and 2.40) and the CH_2(1) (δ(H) 1.88 and 1.50); and the olefinic H-atom H–C(12) and CH_2(11) (δ(H) 1.98). The correlations, in the HMBC experiment, between the H-atoms with the signals at δ(H) 3.63 and 3.38 with the CH group at δ(C) 48.7, assigned to C(5) and bonded to H-atom with a signal at δ(H) 1.99, and between this latter H-atom with the O-bearing CH (δ(C) 68.5) localized an OH group at C(23). Additionally, the HMBCs between the H-atoms resonating at δ(H) 3.63 and 3.38; and Me(26) (δ(H) 0.93) with the CO (δ(C) 219.2); evidencing the C(3)=O group and identifying the Me(23) group; and those between the H-atom with a signal at δ(H) 2.22, bonded to CH, resonating at δ(C) 45.6 and assigned to C(18), with the O-bearing CH with a signal at δ(C) 68.3 indicated an OH group at C(28). The configuration of the OH group at C(16) was delineated by a NOESY experiment, showing NOEs between H–C(16) (δ(H)

4.28) and Me(27) (δ(H) 1.28) and H–C(19), indicating a β-orientation for the OH group.

The ESI-MS of compound **10** showed a *quasi*-molecular-ion peak at *m*/*z* 489.2 ($[M+H]^+$), suggesting the molecular formula $C_{30}H_{48}O_5$ and seven degrees of unsaturation. Fragment-ion peaks at *m*/*z* 471.3 ($[M-H_2O+H]^+$), 453.1 ($[M-2\,H_2O+H]^+$), and a *retro-Diels–Alder* fragmentation-ion peak at *m*/*z* 267.2 (20%) were characteristic for an olean-12-ene derivative. The IR spectrum showed absorption bands at 3350, 1118, 1090, and 1045 cm^{-1}, consistent with the presence of a OH and C–O–C groups, and a band of a CO group at 1724 cm^{-1}. The ^{13}C-NMR spectrum (*Table*) displayed 30 signals, which, with the DEPT experiment, revealed the presence of six Me groups (δ(C) 30.1, 27.7, 18.3, 18.0, 17.7, and 16.3), an olefinic C-atom (δ(C) 124.8), two O-bearing CH groups (δ(C) 74.4 and 68.9), and two O-bearing CH_2 groups (δ(C) 68.4 and 68.3). The ^{1}H-NMR spectrum revealed the presence of six tertiary Me (δ(H) 0.89, 0.93, 0.96, 1.07, 1.10, and 1.28), two O-bearing CH groups (*double doublet*s at δ(H) 4.20 and 3.55), two O-bearing CH_2 groups (*doublet*s at δ(H) 3.60 and 3.78), and it exhibited two signals obscured by the solvent (δ(H) 3.30 and 3.32), and one trisubstituted olefinic H-atom signal at δ(H) 5.31 attributed to H–C(12). Analysis of COSY, NOESY, HSQC, and HMBC spectra allowed us to elucidate the structure of 16β,21β,23,28-tetrahydroxyolean-12-en-3-one for this compound. Many of the correlations observed were similar to those of compound **9**, indicating the same substitution pattern in rings *A*, *B*, *C*, and *D*. Another OH group was assigned to C(21) on the basis of the HMBCs between Me(29) and Me(30) (δ(H) 0.96 and 0.89, resp.) with the O-bearing CH group resonating at δ(C) 74.4, and between H–C(21) (δ(H) 3.55) and the C-atoms resonating at δ(C) 48.6 (C(19)), 45.2 (C(17)), 37.7 (C(20)), 30.1 (C(29)), and 18.0 (C(30)). The configuration of the OH group at C(21) has been determined by a NOESY experiment, where the H-atom with a signal at δ(H) 3.55 showed NOE with Me(29) and H–C(16) revealing a β-orientation for the OH group.

The ESI-MS of compound **11** exhibited a *quasi*-molecular-ion peak at *m*/*z* 505.3 ($[M+H]^+$), suggesting the molecular formula $C_{30}H_{48}O_6$ and seven degrees of unsaturation. Fragment-ion peaks at *m*/*z* 487.2 ($[M-H_2O+H]^+$), 469.4 ($[M-2\,H_2O+H]^+$), and *retro-Diels–Alder* fragmentation-ion peak at *m*/*z* 283.1 (20%) evidenced an olean-12-ene derivative. The IR spectrum showed absorption bands at 3330, 1128, 1080, and 1050 cm^{-1}, consistent with the presence of OH and HO–C groups, and a band of a CO group at 1723 cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra were consistent with an olean-12-ene skeleton. The ^{13}C-NMR spectrum (*Table*) displayed 30 signals, which, with the DEPT experiment, revealed the presence of six Me groups (δ(C) 30.6, 28.1, 19.2, 18.5, 17.8, and 16.3), an olefinic C-atom (δ(C) 125.6), three O-bearing CH groups (δ(C) 78.7, 74.4, and 69.8), and two O-bearing CH_2 groups (δ(C) 69.4 and 59.6). The ^{1}H-NMR spectrum revealed the presence of six tertiary Me (δ(H) 1.30, 1.10, 1.08, 1.00, 0.93, and 0.92), three O-bearing CH groups (one as *double doublet* at δ(H) 4.63) and two as *doublet*s at δ(H) 3.98 and 3.52), two O-bearing CH_2 groups (four *doublet*s at δ(H) 3.92, 3.56 and 3.62, 3.36), and one trisubstituted olefinic H-atom (δ(H) 5.40) assigned as H–C(12). Analysis of COSY, NOESY, HSQC, and HMBC spectra allowed us to determine the structure of 16β,21β,22α,23,28-pentahydroxyolean-12-en-3-one for this compound. Many of the correlations observed were similar to those of compound **10**. The other OH group was assigned to C(22) on the basis of COSY that showed

correlation between H–C(21) and H–C(22) (δ(H) 3.52 and 3.98, resp.), and the large coupling constant of 11.0 Hz indicated their 1,2-*trans* relation. In the HMBC spectrum, correlations between Me(30) (δ(H) 0.92) and Me(29) (δ(H) 1.00) with the O-bearing H–C(21) (δ(C) 78.7); and between H–C(21) and the C-atoms resonating at δ(C) 74.4 (C(22)), 47.5 (C(17)), 30.6 (C(29)), and 19.2 (C(30)) were observed. Furthermore, the O-bearing H–C(22) correlated with C-atoms, resonating at δ(C) 69.8, 59.6, and 43.3, that were assigned to the C(16), C(28), and C(18), respectively. The configuration of the OH groups was determined by a NOESY experiment, where the H–C(21) showed NOE with Me(29) and H–C(16), indicating a β-orientation for the OH group, and H–C(22) showed NOE effects with the Me(30) and H–C(18), revealing an α-orientation for the OH group.

The ESI-MS of compound **12** showed a *quasi*-molecular-ion peak at *m*/*z* 475.1 ($[M+H]^+$), suggesting the molecular formula $C_{30}H_{50}O_4$ and six degrees of unsaturation. Fragment-ion peaks at *m*/*z* 457.3 ($[M-H_2O+H]^+$) and 439.1 ($[M-2\,H_2O+H]^+$) were also observed. The ^{13}C-NMR spectrum (*Table*) showed 30 C-atom signals, identified on the basis of a DEPT experiment as those for five Me, twelve CH_2, seven CH groups, and six quaternary C-atoms. The ^{1}H-NMR data, together with those derived from an HSQC experiment, evidenced the presence of two olefinic H-atoms (δ(H) 4.72 and 4.60), bonded to the C-atom resonating at δ(C) 111.1, four H-atoms (two *doublet*s at δ(H) 4.10 and 3.44, correlated to the C-atom resonating at δ(C) 62.4, and one as *doublet* at δ(H) 3.52, and one at δ(H) 3.29 obscured by solvent, correlated to the C-atom resonating at δ(C) 67.7), and, finally, two H-atoms (*double doublet*s at δ(H) 3.73 and 3.58), assigned to the C-atoms resonating at δ(C) 80.0 and 74.2, respectively. In the upfield region, five Me *singlet*s at δ(H) 1.69, 1.11, 1.03, 0.90, and 0.68, correlated, in the HSQC, with the C-atom resonances at δ(C) 19.9, 17.7, 16.8, 17.5, and 13.0, respectively, were identified. The ^{1}H,^{1}H-COSY experiment showed the following correlations: H–C(3) (δ(H) 3.58) with CH_2(2) (δ(H) 1.66–1.60), which showed a cross-peak with CH_2(1) (δ(H) 1.68–1.63 and 0.95–0.89); H–C(16) (δ(H) 3.73) with CH_2(15) (δ(H) 1.82, 1.45–1.40); and H–C(19) (*multiplet* at δ(H) 2.50–2.45) with H–C(18) (δ(H) 1.58–1.50) and CH_2(21) (δ(H) 2.05–2.02, 1.48–1.41). The HMBC experiment furnished useful data to solve the structure. In fact, the sp^2-C-atom C(29) correlated with Me(30) (δ(H) 1.69) and the H–C(19) (δ(H) 2.50–2.45). The H-atoms of Me(30) were also correlated with the C-atoms resonating at δ(C) 151.6 (C(20)) and 49.6 (C(19)), while H–C(19) also displayed correlations with C-atoms, at resonating δ(C) 46.2, 33.9, and 19.9, assigned to C(17), C(22), and C(30), respectively. Moreover H–C(3) (δ(H) 3.58) and CH_2(23) (δ(H) 3.52, 3.29), in the same HMBC experiment, gave cross-peaks with C-atoms resonating at δ(C) 49.4, 43.9, and 13.0, assigned to C(5), C(4), and C(24), respectively. Finally, H–C(16) (δ(H) 3.73) and CH_2(28) (δ(H) 4.10 and 3.44) gave cross-peaks with C(22) and those, resonating at δ(C) 49.8 and 46.2, assigned to C(18) and C(17), respectively. These data were in accordance with a lupane triterpene structure. The relative configurations of the stereogenic C-atoms were determined by a NOESY experiment. The H-atoms of Me(27) showed NOE with the H–C(16), indicating a β-configuration of the OH group. These data allowed us to determine the structure of 3β,16β,23,28-tetrahydroxylup-20(29)-ene for this compound.

We thank the *Associazione Italiana per la Promozione delle Ricerche su Ambiente e Salute umana* (*AIPRAS-Onlus*) for financial support, and *CIMCF* (Centro Interdipartimentale di Metodologie Chimico-Fisiche) of University of Naples 'Federico II'.

Experimental Part

General. Optical rotations: in MeOH or CH_2Cl_2; *Perkin-Elmer 141* polarimeter. IR Spectra: *Jasco FT/IR–430* instrument. HPLC: *Shimadzu LC-10AD* by using a refractive-index detector *Shimadzu RID-10A*. Semiprep. HPLC: *RP-18* (*LiChrospher* 10 μm, 250 × 10 mm i.d.; *Merck*) column with a flow rate of 1.2 ml min^{-1}. Column chromatography (CC): *Merck Kieselgel 60* (SiO_2; 230–400 mash). Prep. TLC: silica gel (*UV–254* precoated) plates with 0.5- and 1.0-mm thickness (*Merck*). ^1H- and ^{13}C-NMR Spectra: *Varian INOVA-500* FT NMR spectrometer (^1H at 499.710 and ^{13}C at 125.663 MHz), in $CDCl_3$ or CD_3OD solns., at 25°, δ in ppm, J in Hz. Proton-detected heteronuclear correlations were measured using a gradient heteronuclear single-quantum coherence (HSQC), optimized for $^1J(H,C) = 140$ Hz, and a gradient heteronuclear multiple bond coherence (HMBC), optimized for $^nJ(H,C) = 8$ Hz.

Plant Material. Gymnema sylvestre was purchased from *Mother Herbs Ltd.* (13 Street, Madhu Vihar, Patpadganj, Delhi – 110092, India, e-mail: info@motherherbs.com) and identified by Prof. *Antonino Pollio* of the Dipartimento delle Scienze Biologiche of the University of Naples. A sample specimen (HERBNAWY 124) has been deposited with the herbarium of the University Federico II.

Extraction and Isolation. Dried and finely powdered aerial parts of *G. sylvestre* (7.0 kg) were sliced and extracted with H_2O (25 l for 24 h) and successively with CH_2Cl_2 (20 l for 96 h). The org. extract was filtered and evaporated *in vacuo* to remove CH_2Cl_2. The resulting extract (350 g) was fractionated into acidic and neutral fractions with aq. 2N NaOH soln. The neutral soln., washed with H_2O and concentrated *in vacuo* (175 g), was subjected to CC (SiO_2; with petroleum ether (PE), CH_2Cl_2, AcOEt, Me_2CO, MeOH, and H_2O).

The fraction eluted with CH_2Cl_2 (25.0 g) was fractionated by CC (SiO_2; CH_2Cl_2/MeOH 100:0 to 0:100).

The fractions eluted with CH_2Cl_2 (592 mg) were purified by flash CC (SiO_2, and then the fractions eluted with CH_2Cl_2/AcOEt 17:3 (37 mg) were further purified by prep. TLC (CH_2Cl_2/Me_2CO 17:3) to give triterpene **1** (5 mg).

The fractions eluted with CH_2Cl_2/MeOH 19:1 (913 mg) were purified by CC (SiO_2), and the fractions eluted with CH_2Cl_2/MeOH 19:1 (62 mg), were further purified by HPLC (*RP-18*; MeOH/MeCN/H_2O 2:7:1) to yield triterpenes **3**, **4**, **9**, and **13** (2, 2, 15, and 2 mg, resp.).

The fractions eluted with CH_2Cl_2/MeOH 9:1 (96 mg) were purified by HPLC (*RP-18*; MeOH/MeCN/H_2O 3:4:3) to yield triterpene **6** (36 mg).

The fractions eluted with CH_2Cl_2/MeOH 9:1 (2.28 g) were purified by CC (SiO_2), and then fractions eluted with CH_2Cl_2/MeOH 4:1 (291 mg) were purified by HPLC (*RP-18*; MeOH/MeCN/H_2O 2:2:1) to give triterpenes **8** and **12** (3 and 15 mg, resp.).

The fractions eluted with CH_2Cl_2/MeOH 1:9 (6.38 g) were purified by flash CC (SiO_2), and the fractions eluted with Me_2CO (611 mg) were purified by HPLC (*RP-18 Sep–Pak*; MeOH/MeCN/H_2O 1:2:2) to give *Frs. 1–6*. *Fr. 4* (60 mg) was purified HPLC (*RP-18*; MeOH/MeCN/H_2O 2:3:5) to give triterpene **11** (6 mg). *Fr. 5* (127 mg) was purified by HPLC (*RP-18*; MeOH/MeCN/H_2O 2:5:3) to give triterpenes **2**, **5**, and **7** (43, 12, and 10 mg, resp.). *Fr. 6* (25 mg) was purified by HPLC (*RP-18*; MeOH/MeCN/H_2O 2:5:3) to give triterpene **10** (22 mg).

3β,16β,21β,23-Tetrahydroxyolean-12-ene (*=(3β,16β,21β)-Olean-12-ene-3,16,21,23-tetrol*; **5**). Amorphous powder. $[\alpha]_D^{25} = +20.5$ ($c = 0.27$, MeOH). IR (film): 3347, 1130, 1080, 1035. ^1H-NMR (CD_3OD): 5.28 (br. *s*, H–C(12)); 4.10 (*dd*, $J = 11.7, 3.9$, H–C(16)); 3.61 (*dd*, $J = 11.0, 4.4$, H–C(3)); 3.53 (*d*, $J = 11.3$, H_a–C(23)); 3.52 (*dd*, $J = 10.8, 4.2$, H–C(21)); 3.31 (obscured by solvent, H_b–C(23)); 2.20 (*dd*, $J = 14.1, 3.8$, H–C(18)); 2.10 (br. *d*, $J = 13.1$, CH_2(19)); 1.98–1.93 (*m*, CH_2(2), CH_2(11)); 1.70–1.60 (*m*, CH_2(22)); 1.71–1.56 (*m*, 1 H of CH_2(7)); 1.69–1.59 (*m*, 1 H of CH_2(15)); 1.69–1.61 (*m*, 1 H of CH_2(1)); 1.65–1.59 (*m*, H–C(5)); 1.50–1.42 (*m*, CH_2(6)); 1.33–1.27 (*m*, 1 H of CH_2(7)); 1.27–1.21 (*m*, 1 H of CH_2(15)); 1.23 (*s*, Me(27)); 1.20–1.15 (*m*, H–C(9)); 1.18–1.11 (*m*, 1 H of CH_2(1)); 1.02 (*s*, Me(26)); 1.01 (*s*, Me(25));

0.95 (*s*, Me(29)); 0.87 (*s*, Me(30)); 0.81 (*s*, Me(28)); 0.71 (*s*, Me(24)). ^{13}C-NMR (CD_3OD): see the *Table*. ESI-MS: 475.2 ($[M+H]^+$). HR-ESI-MS: 475.3769 ($[M+H]^+$, $C_{30}H_{51}O_4^+$; calc. 475.3787).

3β,16β,21α,23,28-Pentahydroxyolean-12-ene (*=(3β,16β,21α)-Olean-12-ene-3,16,21,23,28-pentol*; **7**). Amorphous powder. $[\alpha]_D^{25} = +32.3$ ($c = 0.23$, MeOH). IR (film): 3330, 1115, 1095, 1035. ^{1}H-NMR (CD_3OD): 5.29 (br. *s*, H–C(12)); 4.57 (*dd*, *J* = 11.7, 3.9, H–C(16)); 3.74 (*d*, *J* = 10.8, H_a–C(28)); 3.62 (*dd*, *J* = 11.0, 4.4, H–C(3)); 3.54 (*d*, *J* = 11.3, H_a–C(23)); 3.52 (br. *t*, *J* = 3.0, H–C(21)); 3.32 (obscured by solvent, H_b–C(28), H_b–C(23)); 2.30 (*dd*, *J* = 14.1, 3.8, H–C(18)); 2.17 (br. *d*, *J* = 13.1, CH_2(19)); 2.16–2.10 (*m*, 1 H of CH_2(22)); 1.99–1.94 (*m*, CH_2(11)); 1.82–1.74 (*m*, 1 H of CH_2(15)); 1.79–1.70 (*m*, 1 H of CH_2(22)); 1.73–1.67 (*m*, CH_2(2)); 1.71–1.66 (*m*, 1 H of CH_2(7)); 1.69–1.62 (*m*, 1 H of CH_2(1)); 1.66–1.61 (*m*, H–C(9)); 1.52–1.43 (*m*, 1 H of CH_2(6)); 1.41–1.34 (*m*, 1 H of CH_2(7)); 1.41–1.33 (*m*, 1 H of CH_2(6)); 1.33–1.25 (*m*, 1 H of CH_2(15)); 1.26 (*s*, Me(27)); 1.22–1.15 (*m*, H–C(5)); 1.05–1.00 (*m*, 1 H of CH_2(1)); 1.03 (*s*, Me(26)); 1.01 (*s*, Me(25)); 0.95 (*s*, Me(29)); 0.92 (*s*, Me(30)); 0.71 (*s*, Me(24)). ^{13}C-NMR (CD_3OD): see the *Table*. ESI-MS: 491.4 ($[M+H]^+$). HR-ESI-MS: 491.3728 ($[M+H]^+$, $C_{30}H_{51}O_5^+$; calc. 491.3736).

3β,16β,23,28-Tetrahydroxyolean-13(18)-ene (*=(3β,16β)-Olean-13(18)-ene-3,16,23,28-tetrol*; **8**). Amorphous powder. $[\alpha]_D^{25} = -1.2$ ($c = 0.19$, MeOH). IR (film): 3334, 1112, 1087, 1034. ^{1}H-NMR (CD_3OD): 3.82 (*d*, *J* = 11.4, H_a–C(28)); 3.76 (*d*, *J* = 11.4, H_b–C(28)); 3.72 (*dd*, *J* = 12.8, 4.1, H–C(16)); 3.62 (*dd*, *J* = 10.9, 4.9, H–C(3)); 3.54 (*d*, *J* = 11.7, H_a–C(23)); 3.30 (obscured by solvent, H_b–C(23)); 2.72 (br. *d*, *J* = 15.0, 1 H of CH_2(12)); 2.35 (*d*, *J* = 14.1, 1 H of CH_2(19)); 2.33–2.28 (*m*, CH_2(21)); 1.98 (br. *t*, *J* = 12.8, H–C(15)); 1.89 (br. *t*, *J* = 15.7, 1 H of CH_2(12)); 1.73–1.66 (*m*, 1 H of CH_2(1)); 1.68–1.63 (*m*, H–C(19)); 1.65–1.59 (*m*, CH_2(2)); 1.65–1.56 (*m*, CH_2(21)); 1.62–1.54 (*m*, H–C(9)); 1.51–1.45 (*m*, 1 H of CH_2(6)); 1.38–1.33 (*m*, 1 H of CH_2(6)); 1.37–1.30 (*m*, 1 H of CH_2(15)); 1.27 (*m*, CH_2(11)); 1.26 (*s*, Me(27)); 1.24 (*m*, CH_2(7)); 1.20–1.15 (*m*, H–C(5)); 1.06–1.01 (*m*, 1 H of CH_2(1)); 0.96 (*s*, Me(29)); 0.92 (*s*, Me(25), Me(26)); 0.76 (*s*, Me(30)); 0.68 (*s*, Me(24)). ^{13}C-NMR (CD_3OD): see the *Table*. ESI-MS: 475.5 ($[M+H]^+$). HR-ESI-MS: 475.3745 ($[M+H]^+$, $C_{30}H_{51}O_4^+$; calc. 475.3787).

16β,23,28-Trihydroxyolean-12-en-3-one (**9**). Amorphous powder. $[\alpha]_D^{25} = +25.5$ ($c = 0.21$, MeOH). IR (film): 3376, 1722, 1118, 1090, 1045. ^{1}H-NMR (CD_3OD): 5.29 (br. *s*, H–C(12)); 4.28 (*dd*, *J* = 12.0, 4.8, H–C(16)); 3.87 (*d*, *J* = 10.0, H_a–C(28)); 3.63 (*d*, *J* = 10.0, H_a–C(23)); 3.38 (obscured by solvent, H_b–C(23)); 3.29 (oscured by solvent, H_b–C(28)); 2.49–2.40 (*m*, CH_2(2)); 2.24–2.19 (*m*, H–C(18)); 2.18–2.12 (*m*, 1 H of CH_2(22)); 2.02–1.75 (*m*, CH_2(19)); 1.99–1.96 (*m*, H–C(5)); 1.99–1.94 (*m*, CH_2(11)); 1.89–1.83 (*m*, 1 H of CH_2(1)); 1.89–1.85 (*m*, 1 H of CH_2(15)); 1.76–1.71 (*m*, 1 H of CH_2(7)); 1.77–1.72 (*m*, H–C(9)); 1.52–1.45 (*m*, CH_2(6)); 1.51–1.48 (*m*, 1 H of CH_2(1)); 1.45–1.39 (*m*, 1 H of CH_2(22)); 1.45–1.38 (*m*, 1 H of CH_2(21)); 1.44–1.40 (*m*, 1 H of CH_2(15)); 1.43–1.39 (*m*, 1 H of CH_2(7)); 1.28 (*s*, Me(27)); 1.25–1.20 (*m*, 1 H of CH_2(21)); 1.11 (*s*, Me(26)); 1.07 (*s*, Me(25)); 0.98 (*s*, Me(29)); 0.93 (*s*, Me(24), Me(30)). ^{13}C-NMR (CD_3OD): see the *Table*. ESI-MS: 473.2 ($[M+H]^+$). HR-ESI-MS: 473.3620 ($[M+H]^+$, $C_{30}H_{49}O_4^+$; calc. 473.3631).

16β,21β,23,28-Tetrahydroxyolean-12-en-3-one (**10**). Amorphous powder. $[\alpha]_D^{25} = +26.5$ ($c = 0.22$, MeOH). IR (film): 3350, 1724, 1118, 1090, 1045. ^{1}H-NMR (CD_3OD): 5.31 (br. *s*, H–C(12)); 4.20 (*dd*, *J* = 11.7, 4.8, H–C(16)); 3.78 (*d*, *J* = 10.8, H_a–C(28)); 3.60 (*d*, *J* = 10.8, H_a–C(23)); 3.55 (*dd*, *J* = 11.7, 3.90, H–C(21)); 3.32 (oscured by solvent, H_b–C(28)); 3.30 (oscured by solvent, H_b–C(23)); 2.33–2.27 (*m*, H–C(18)); 2.30–1.50 (*m*, CH_2(22)); 2.00 (*m*, CH_2(11)); 1.98 (br. *d*, *J* = 12.3, 1 H of CH_2(15)); 1.92–1.86 (*m*, 1 H of CH_2(1)); 1.89–1.75 (*m*, 1 H of CH_2(7)); 1.88–1.82 (*m*, 1 H of CH_2(2)); 1.88–1.80 (*m*, H–C(9)); 1.83–1.78 (*m*, 1 H of CH_2(6)); 1.82–1.77 (*m*, 1 H of CH_2(19)); 1.75–1.70 (*m*, H–C(5)); 1.52–1.47 (*m*, 1 H of CH_2(1)); 1.50–1.45 (*m*, 1 H of CH_2(7)); 1.40–1.35 (*m*, 1 H of CH_2(2)); 1.40–1.31 (*m*, 1 H of CH_2(6)); 1.33–1.28 (*m*, 1 H of CH_2(19)); 1.28 (br. *d*, *J* = 12.3, 1 H of CH_2(15)); 1.28 (*s*, Me(27)); 1.10 (*s*, Me(26)); 1.07 (*s*, Me(25)); 0.96 (*s*, Me(29)); 0.93 (*s*, Me(24)); 0.89 (*s*, Me(30)). ^{13}C-NMR (CD_3OD): see the *Table*. ESI-MS: 489.2 ($[M+H]^+$). HR-ESI-MS: 489.3563 ($[M+H]^+$, $C_{30}H_{49}O_5^+$; calc. 489.3580).

16β,21β,22α,23,28-Pentahydroxyolean-12-en-3-one (**11**). Amorphous powder. $[\alpha]_D^{25} = +23.1$ ($c = 0.27$, MeOH). IR (film): 3330, 1723, 1128, 1080, 1050. ^{1}H-NMR (CD_3OD): 5.39 (*t*, *J* = 3.4, H–C(12)); 4.63 (*dd*, *J* = 11.6, 5.2, H–C(16)); 3.98 (*d*, *J* = 11.0, H–C(22)); 3.92 (*d*, *J* = 10.9, H_a–C(28)); 3.62 (*d*, *J* = 10.8, H_a–C(23)); 3.56 (*d*, *J* = 10.9, H_b–C(28)); 3.52 (*d*, *J* = 11.0, H–C(21)); 3.36 (*d*, *J* = 10.8, H_b–C(23)); 2.68 (*dd*, *J* = 14.2, 4.3, H–C(18)); 2.50, 2.38 (2*m*, CH_2(2)); 2.05 (*m*, 1 H of CH_2(11)); 1.98 (*m*, H–C(9)); 1.96 (*m*, 1 H of CH_2(11)); 1.92 (*m*, 1 H of CH_2(19)); 1.90, 1.50 (2*m*, CH_2(1)); 1.77 (*dd*, *J* = 11.5, 6.1, H–C(5));

1.71 (*m*, 1 H of CH_2(7)); 1.68 (br. *d*, *J* = 12.3, 1 H of CH_2(15)); 1.48 (*m*, CH_2(6)); 1.36 (br. *d*, *J* = 12.7, 1 H of CH_2(7)); 1.30 (*s*, Me(27)); 1.26 (*m*, 1 H, Me(15)); 1.20 (*dd*, *J* = 14.0, 4.5, 1 H of CH_2(19)); 1.10 (*s*, Me(26)); 1.08 (*s*, Me(25)); 1.00 (*s*, Me(29)); 0.93 (*s*, Me(24)); 0.92 (*s*, Me(30)). ^{13}C-NMR (CD_3OD): see the *Table*. ESI-MS: 505.3 ($[M+H]^+$). HR-ESI-MS: 505.3500 ($[M+H]^+$, $C_{30}H_{49}O_6^+$; calc. 505.3529).

3β,16β,23,28-Tetrahydroxylup-20(29)-ene (*=(3β,16β)-Lup-20(29)-ene-3,16,23,28-tetrol*; **12**). Amorphous powder. $[\alpha]_D^{25} = +10.5$ (*c* = 0.23, MeOH). IR (film): 3350, 1556, 1090, 1045. ^{1}H-NMR (CD_3OD): 4.72 (br. *s*, H–C(29)); 4.60 (br. *s*, H–C(29)); 4.10 (*d*, *J* = 11.5, H_a–C(28)); 3.73 (*dd*, *J* = 10.5, 5.0, H–C(16)); 3.58 (*dd*, *J* = 11.0, 5.0, H–C(3)); 3.52 (*d*, *J* = 11.5, H_a–C(23)); 3.44 (*d*, *J* = 11.5, H_b–C(28)); 3.29 (overlapped, H_b–C(23)); 2.50–2.45 (*m*, H–C(19)); 2.37 (br. *dd*, *J* = 12.5, 7.9, 1 H of CH_2(22)); 2.05–2.02 (*m*, 1 H of CH_2(21)); 1.82 (br. *t*, *J* = 12.2, 1 H of CH_2(15)); 1.69 (*s*, Me(30)); 1.71–1.63 (*m*, 1 H of CH_2(12)); 1.68–1.63 (*m*, 1 H of CH_2(1)); 1.66–1.60 (*m*, CH_2(2)); 1.59–1.40 (*m*, CH_2(7)); 1.58–1.50 (*m*, H–C(18)); 1.48–1.41 (*m*, 1 H of CH_2(21), CH_2(6)); 1.46–1.23 (*m*, CH_2(11)); 1.45–1.40 (*m*, 1 H of CH_2(15)); 1.38–1.31 (*m*, H–C(9)); 1.22–1.17 (*m*, 1 H of CH_2(22)); 1.11 (*s*, Me(26)); 1.12–1.07 (*m*, H–C(5)); 1.05–1.01 (*m*, 1 H of CH_2(12)); 1.03 (*s*, Me(27)); 0.95–0.89 (*m*, 1 H of CH_2(1)); 0.90 (*s*, Me(25)); 0.68 (*s*, Me(24)). ^{13}C-NMR (CD_3OD): see the *Table*. ESI-MS: 475.1 ($[M+H]^+$). HR-ESI-MS: 475.3765 ($[M+H]^+$, $C_{30}H_{51}O_4^+$; calc. 475.3787).

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Received April 5, 2012