Anti-AIDS Agents. 30. Anti-HIV Activity of Oleanolic Acid, Pomolic Acid, and Structurally Related Triterpenoids[1]

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Oleanolic acid (**1**) was identified as an anti-HIV principle from several plants, including *Rosa woodsii* (leaves), *Prosopis glandulosa* (leaves and twigs), *Phoradendron juniperinum* (whole plant), *Syzygium claviflorum* (leaves), *Hyptis capitata* (whole plant), and *Ternstromia gymnanthera* (aerial part). It inhibited HIV-1 replication in acutely infected H9 cells with an EC_{50} value of 1.7 μg/mL, and inhibited H9 cell growth with an IC_{50} value of 21.8 μg/mL [therapeutic index (T. I.) 12.8]. Pomolic acid, isolated from *R. woodsii* and *H. capitata*, was also identified as an anti-HIV agent (EC_{50} 1.4 μg/mL, T. I. 16.6). Although ursolic acid did show anti-HIV activity (EC_{50} 2.0 μg/mL), it was slightly toxic (IC_{50} 6.5 μg/mL, T. I. 3.3). A new triterpene (**11**) was also isolated from the $CHCl_3$-soluble fraction of *R. woodsii*, though it showed no anti-HIV activity. The structure of **11** was determined to be 1β-hydroxy-2-oxopomolic acid by spectral examination. Based on these results, we examined the anti-HIV activity of oleanolic acid- or pomolic acid-related triterpenes isolated from several plants. In addition, we previously demonstrated that derivatives of betulinic acid, isolated from the leaves of *S. claviflorum* as an anti-HIV principle, exhibited extremely potent anti-HIV activity. Accordingly, we prepared derivatives of oleanolic acid and evaluated their anti-HIV activity. Among the oleanolic acid derivatives, **18** demonstrated most potent anti-HIV activity, with an EC_{50} value of 0.0005 μg/mL and a T. I. value of 22 400.

We are continuing a search for new anti-HIV agents from natural products, and also modification of the active principles to develop more potent anti-HIV agents.[2] As a part of our continuing screening of plant extracts as anti-HIV agents, the MeOH extracts of the leaves of *Rosa woodsii* Lindl. (Rosaceae), the leaves and twigs of *Prosopis glandulosa* Torr. (Leguminosae), the whole plant of *Phoradendron juniperinum* Engel. (Loranthaceae), the leaves of *Syzygium claviflorum* Wall. ex A. & J. Cowan (Myrtaceae), the whole plant of *Hyptis capitata* Jacq. (Labiatae), and the aerial parts of *Ternstromia gymnanthera* Sprague (Theaceae) demonstrated significant anti-HIV activity (EC_{50} < 20 μg/mL). Subsequent bioassay-directed fractionation of these extracts has resulted in the isolation of oleanolic acid (**1**) as an anti-HIV principle from all of the foregoing plants. Pomolic acid (**5**), isolated from *R. woodsii* and *H. capitata*, was also identified as an anti-HIV agent. Based on these results, we have examined anti-HIV activity of oleanolic acid- or pomolic acid-related triterpenes isolated from several plants. Furthermore, a series of oleanolic acid derivatives has been prepared and their anti-HIV activity evaluated.

Results and Discussion

In the course of our continuing screening of plant extracts as potential anti-HIV agents, the MeOH extracts of the leaves of *R. woodsii* (Rosaceae), the leaves and twigs of *P. glandulosa* (Leguminosae), the whole plant of *Ph. juniperinum* (Loranthaceae), the leaves of *S. claviflorum* (Myrtaceae), the whole plant of *H. capitata* (Labiatae), and the aerial part of *T. gymnanthera* (Theaceae) demonstrated significant anti-HIV activity (EC_{50} < 20 μg/mL) consistent with the presence of an active substance(s). Subsequent bioassay-directed solvent extraction of these extracts successively with *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH yielded a $CHCl_3$-soluble anti-HIV active fraction in each case. Further bioassay-guided chromatography of active fractions resulted in the isolation of oleanolic acid (**1**) as an anti-HIV principle from all of these plant extracts. Pomolic acid (**5**), isolated from *R. woodsii* and *H. capitata*, was also identified as an anti-HIV triterpene, while betulinic acid (**15**), a previously reported anti-HIV triterpene, was

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Table 1. Anti-HIV Activities for Oleanolic Acid (**1**), Pomolic Acid (**5**), and Related Triterpenes

compound	IC_{50} (μg/mL)[a]	EC_{50} (μg/mL)[b]	T. I.[c]
1	21.8 ± 13.6	1.7 ± 1.7	12.8
2	8	NS[d]	
3	36	15	2.4
4	25	4.4	5.7
5	23.3 ± 16.6	1.4 ± 0.1	16.6
6	6.5 ± 1.9	2.0 ± 1.0	3.3
7	8	4	2
8	9	NS	
9	15	NS	
10	40	20	2
11	40	NS	
12	>20[e]	NS	
13	37.5 ± 2.9	NS	
14	>100[f]	35	>2.9
16	4	20	0.25
18	11.2	0.0005	22 400
19	9.7	NS	
20	14.2	1.5	9.3
21	22.1	NS	
22	6.5 ± 3.1	6.1 ± 4.0	1.1
23	14.6	7.1	2.1
24	27.5	8.3	3.3
25	4.4	1.1	4.1
26	4.7	1.2	3.9
27	0.8	0.11	7.3
28	34.3 ± 12.5	0.5 ± 0.26	68.6
AZT	500	0.012	41 667
	1871 μM	0.045 ± 0.056[g]	41 667

[a] The agent concentration that inhibited H9 cell growth by 50%. [b] The agent concentration that inhibited viral replication in H9 cell by 50%. [c] In vitro therapeutic index (TI) radio: IC_{50}/EC_{50}. [d] NS = no suppression. [e] Due to limited quantities, this agent could not be tested at 100 μg/mL concentration; the maximum concentration tested in the presence of DMSO was 20 μg/mL. [f] Because all agents were dissolved in DMSO and their stock concentrations were 10 mg/mL, a minimum of a 1:100 dilution was needed to negate the effects of DMSO on this assay; thus, the greatest agent concentration possible was 100 μg/mL. [g] This EC_{50} value represents the mean and standard deviation of 65 EC_{50} values for AZT.

isolated from *R. woodsii* and *T. gymnanthera*. Oleanolic acid (**1**) and pomolic acid (**5**) demonstrated anti-HIV activity, with EC_{50} values of 1.7 and 1.4 μg/mL, respectively, and inhibited uninfected H9 cell growth with IC_{50} values of 21.8 and 23.3 μg/mL, respectively. Their calculated therapeutic index (T. I.) values [defined as toxicity (IC_{50}) divided by anti-HIV activity (EC_{50})] were 12.8 and 16.6, respectively. Ursolic acid (**6**), isolated from *P. glandulosa*, *S. claviflorum*, and *H. capitata*, was found to show similar anti-HIV activity, with an EC_{50} value of 2.0 μg/mL and IC_{50} values of 6.5 μg/mL (T. I. value, 3.3).

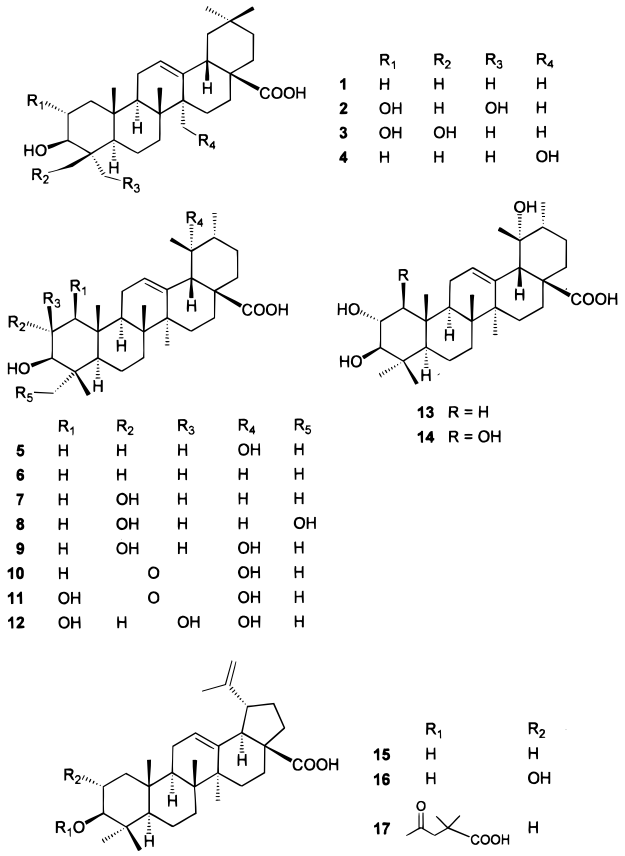
The HIV inhibitory effects of 11 oleanolic acid- and pomolic acid-related triterpenes, isolated from various plant sources,[4,5,6,12] were examined. Among the compounds tested, only oleanolic acid and pomolic acid exhibited anti-HIV activity as shown in Table 1. The compounds with additional oxygen function(s) to those in oleanolic acid or pomolic acid showed decreased anti-HIV activity. Alphitolic acid (**16**), isolated from *R. woodsii*, has the same structure as betulinic acid (**15**) except for an additional hydroxy group at C-2; it also showed decreased anti-HIV activity, which was similar to observations found for oleanolic acid- and pomolic acid-related compounds.

A new triterpenoid (**11**) was also obtained from the $CHCl_3$-soluble fraction of *R. woodsii* by repeated Si gel and ODS chromatography, although it showed no anti-HIV activity. The ^{13}C NMR spectrum of **11** was similar to that of 2-oxopomolic acid (**10**),[9] except for the presence of an oxygen-bearing methine carbon signal at δ 85.3 as well as the absence of one methylene carbon signal. The ^1H NMR spectrum also resembled that of **10**, but it revealed an additional one-proton singlet at δ 4.37 due to a hydroxy-carrying methine signal. The EIMS of **11** gave an M^+ ion at *m/z* 502, and the molecular formula was confirmed as $C_{30}H_{45}O_6$ by HRFABMS. The molecular weight was 16 mass units more than that of **10**. Treatment of **11** with Ac_2O and pyridine yielded a diacetate with an M^+ ion peak at *m/z* 586. These observations indicate that **11** possesses an additional hydroxy group compared to **10**. The location of the additional hydroxy group was determined to be at C-1 by examination of HMBC; the methine proton signal at δ 4.37 showed long-range correlations with the C-2 carbonyl (δ 212.2), C-9 methine (δ 48.7), and 26-CH_3 (δ 17.1) carbon signals, and a long-range correlation between H-25 (δ 0.96) and the carbon resonance at δ 85.3 was observed. The configurations of the hydroxy groups at C-1 and C-3 were concluded to be β by observation of NOEs between H-5 and H-1 as well as H-3 in an NOE difference experiment. On the basis of these spectral examinations, the structure of **11** was concluded to be 1β-hydroxy-2-oxopomolic acid.

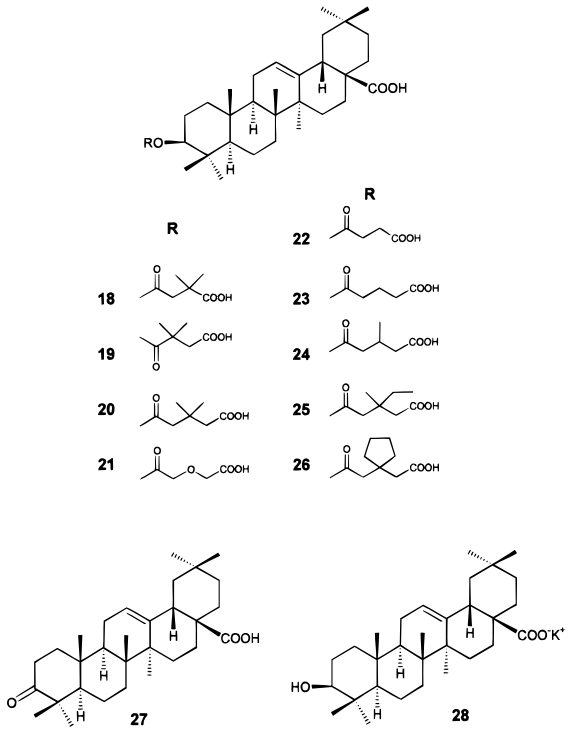
We have reported previously that modification of betulinic acid (**15**), identified as the anti-HIV principle of the leaves of *S. claviflorum*, yielded extremely potent anti-HIV compounds, including **17**, with an EC_{50} value of < 0.000 35 μM and a T. I. of > 20 000.[2] Because the anti-HIV activity of oleanolic acid (**1**) was comparable to that of betulinic acid, oleanolic acid was considered to be a potential anti-HIV lead triterpenoid. Based on the these findings, derivatives of oleanolic acid were prepared and evaluated for their inhibitory effect on HIV replication. The structures of oleanolic acid and betulinic acid are partially correlated, especially in the A- through D-ring moiety, but differ mainly in the E-ring moiety. Therefore, modification of oleanolic acid focused on introducing an ester group at the C-3 hydroxy group, based on the structures of potent anti-HIV betulinic acid derivatives.

As seen in previous betulinic acid derivatives, oleanolic acid 3-*O*-3′,3′-dimethylsuccinate (**18**) demonstrated a most potent anti-HIV activity, with an EC_{50} value of 0.0005 μg/mL, and showed a greatly improved T. I. value (22 400), while the lack of any discernible anti-HIV activity in its isomer (**19**) was similar to the corresponding betulinic acid derivative. Compounds **20**, **25**, and **26** displayed relatively potent anti-HIV activity, with EC_{50} values of 1.5, 1.1, and 1.2 μg/mL, respectively, but were not as potent as **18**. Moreover, the small T. I. values of **25** and **26** (4.1 and 3.9, respectively) suggested that they are slightly toxic.

Oxidation of oleanolic acid (**1**) with pyridinium chlorochromate (PCC) yielded a 3-oxo- derivative (**27**), which was more toxic against uninfected H9 cells than its parent compound, but inhibited HIV-1 replication, with an improved EC_{50} value of 0.11 μg/mL. Treatment of **1** with a molar equivalent of potassium hydroxide furnished the potassium salt of oleanolic acid (**28**). Compound **28** exhibited potent anti-HIV activity, with an EC_{50} value of 0.5 μg/mL and a T.I value of 68.6. Such enhanced anti-HIV activity with the potassium salt



compared to the free carboxylic acid was also found in caffeic acid tetramers.[3]



Experimental Section

General Experimental Procedures. Melting points were measured on a Fisher−Johns or a Yanako micromelting-point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-360 or DIP-1000 polarimeter. MS were determined on a JEOL HX-110 spectrometer. ^{1}H and ^{13}C NMR spectra were measured on Bruker AC-300, JEOL A-400, and JEOL A-500 spectrometers with TMS as internal standard. All known triterpene acids were identified by comparison with reported physical and spectral data.

Table 2. Anti-HIV Activities for MeOH Extract and Fractions from *Ph. juniperinum*

	IC_{50} (μg/mL)	EC_{50} (μg/mL)
MeOH extract	100	> 4 but < 20
n-hexane-soluble fraction	100	4
$CHCl_3$-soluble fraction	> 20 but < 100	< 0.8
Me_2CO-soluble fraction	100	11
MeOH-soluble fraction	> 100	> 100
fraction C-1	> 100	4
fraction C-2	> 4 but < 20	6
fraction C-3	> 100	20
fraction C-4	> 20 but < 100	10
fraction C-5	20	0.8
fraction C-6	> 20 but < 100	> 1 but < 5
fraction C-7	> 20 but < 100	> 3 but < 15

Plant Materials. *R. woodsii* was collected at Aspen Grove, Mt. Timpanogos, Utah, in August 1990 and 1991. *Ph. juniperinum* was collected at Blanding, Utah, in April 1993, and *P. glandulosa* was collected at Witchita Mountains Wild Refuge, Oklahoma, in May 1992. Voucher specimens of *R. woodsii* and *Ph. juniperinum* are deposited in the herbarium of Monte L. Bean Life Science Museum, Brigham Young University, Provo, Utah. The voucher specimen of *P. glandulosa* is deposited in the Natural Products Laboratory, School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina. *Sy. claviflorum* was collected in Lanyu, Taiwan, in February 1990. A voucher specimen is deposited in the School of Pharmacy, Kaohsiung Medical College, Taiwan. *T. gymnanthera* was collected in the People's Republic of China. The extraction and isolation of hyptatic acid A (**2**), 2α-hydroxy-ursolic acid (**7**), ursolic acid (**6**), and tormentic acid (**9**) from *Hyptis capitata* have been reported.[4,5]

Bioassay-Guided Fractionation and Isolation. Fractionation and isolation were guided by the anti-HIV assay results. A typical example is described here for the bioassay-guided fractionation and isolation of oleanolic acid (**1**) from *Ph. juniperinum*. The dried whole plant of *Ph. juniperinum* (4.5 kg) was extracted with MeOH, and the MeOH extract (750 g) was adsorbed on Celite. The Celite−MeOH mixture was washed successively with *n*-hexane, $CHCl_3$, Me_2CO, and MeOH. The $CHCl_3$-soluble fraction (70 g) was chromatographed on Si gel using $CHCl_3$−MeOH (1:0 → 9:1) to give seven fractions (fractions C-1−C-7). Fraction C-5 consisted mainly of oleanolic acid, which was purified by Si gel chromatography with C_6H_6−EtOAc (10:1 → 3:1) as eluent to afford pure compound **1** (12.6 g). The anti-HIV activities for the MeOH extract and each fraction from *P. juniperinum* are shown in Table 2.

Isolation of Oleanolic Acid (1), Arjunolic Acid (3), Ursolic Acid (6), Asiantic Acid (8), and Betulinic Acid (15) from *Sy. claviflorum*. The dried leaves (3.0 kg) of *Sy. claviflorum* were extracted with MeOH, and a portion (120g) of this MeOH extract (315 g total) was suspended in H_2O and partitioned with hexane, $CHCl_3$, EtOAc, and BuOH, successively. The $CHCl_3$ extract (25.5 g) was chromatographed on Si gel using $CHCl_3$−EtOAc (4:1) and $CHCl_3$−MeOH (95:5) as

eluents to afford fractions 1−6. Fraction 2 was chromatographed on MCI gel CHP-20P (90 → 100% MeOH) and reversed-phase Si gel (ODS) (90% MeOH in 1% CH_3COOH) to give oleanolic acid (**1**) (42 mg), ursolic acid (**6**)[5] (62 mg), and betulinic acid (**15**)[6] (612 mg). Fraction 5 was chromatographed on MCI gel CHP-20P (80 → 100% MeOH) and ODS (60 → 100% MeOH) to yield a mixture (480 mg) of arjunolic acid (**3**)[7] and asiatic acid (**8**).[7] The mixture was dissolved and stirred in pyridine (3 mL) and Ac_2O (3 mL) at room temperature for 24 h. The solvent was evaporated, and the residue was chromatographed on ODS (85% MeOH in 1% CH_3COOH) to give the acetates of **3** and **8**. Both acetates were treated with 2% NaOMe−MeOH at room temperature for 1 day followed by neutralization with Amberlite IR-120B to afford the deacylated products, **3** (262 mg) and **8** (122 mg).

Isolation of Oleanolic Acid (1), Pomolic Acid (5), Tormentic Acid (9), 2-Oxopomolic Acid (10), 1*β*-Hydroxy-2-oxopomolic Acid (11), 1*β*,2*β*-Dihydroxypomolic Acid (12), Euscaphic Aid (13), 1*β*-Hydroxyeuscaphic Acid (14), Betulinic Acid (15), and Alphitolic Acid (16) from *R. woodsii*. The dried leaves of *R. woodsii* (2.3 kg) were extracted with MeOH, and the MeOH extract (390 g) was adsorbed on Celite. The Celite−MeOH mixture was washed successively with hexane, $CHCl_3$, Me_2CO, and MeOH. The $CHCl_3$-soluble fraction (22 g) was chromatographed on Si gel to give fractions 1−7. Fraction 1 was purified by Si gel column chromatography (C_6H_6) to give 3-*O*-acetyl pomolic acid (14 mg). Fractions 2 and 3 were combined and chromatographed on Si gel (C_6H_6−EtOAc, 19:1 → 9:1) to yield oleanolic acid (**1**) (15 mg) and betulinic acid (**15**)[6] (8 mg). Fraction 4 was chromatographed on Si gel ($CHCl_3$−MeOH, 1:0 → 19:1) and ODS (75 → 85% MeOH) to give oleanolic acid (**1**) (137 mg), pomolic acid (**5**)[8] (33 mg), and 2-oxopomolic acid (**10**)[9] (54 mg). Fractions 5 and 6 were also combined and repeatedly chromatographed on ODS (65 → 75% MeOH) and finally purified by Si gel column chromatography ($CHCl_3$−MeOH, 1:0 → 19:1) to afford tormentic acid (**9**)[8] (72 mg), euscaphic acid (**13**)[8] (124 mg), 1*β*-hydroxyeuscaphic acid (**14**)[10] (21 mg), 1*β*,2*β*-dihydroxypomolic acid (**12**) (20 mg),[11] and a new triterpene acid (**11**) (24 mg).

1*β*-Hydroxy-2-oxopomolic acid (1*β*,3*β*,19α-trihydroxy-2-oxours-12-en-28-oic acid) (11): a white crystalline powder from hexane−Et_2O; mp 227−229 °C; $[\alpha]^{23}_D$ −105.6 °C (*c* 0.26, MeOH); IR (KBr) ν_{max} 3570, 3480, 2930, 1715, 1690 cm^{-1}; ^1H NMR (pyridine-d_5) δ 0.90 (3H, s, H-24), 0.96 (3H, s, H-25), 1.13 (3H, d, J = 7 Hz, H-30), 1.14 (3H, s, H-26), 1.35 (3H, s, H-23), 1.45 (3H, s, H-29), 1.80 (3H, s, H-27), 3.07 (1H, s, H-18), 3.16 (1H, m, H-16a), 4.33 (1H, s, H-3), 4.37 (1H, s, H-1), 5.70 (1H, t-like, H-12); ^{13}C NMR δ (pyridine-d_5) 12.5 (C-25), 16.8 (C-30), 17.1 (C-24 and 26), 18.7 (C-6), 24.7 (C-27), 26.4 (C-16), 27.0 (C-21), 27.1 (C-29), 27.3 (C-11), 29.4 (C-23), 29.5 (C-15), 33.4 (C-7), 38.5 (C-22), 41.2 (C-8), 42.2 (C-14), 42.4 (C-20), 45.8 (C-4), 48.3 (C-17), 48.7 (C-9), 49.6 (C-10), 51.9 (C-5), 54.6 (C-18), 72.7 (C-19), 81.9 (C-3), 85.3 (C-1), 129.1 (C-12), 138.9 (C-13), 180.7 (C-28), 212.2 (C-2); EIMS *m/z* 502 (M^+), 484, 466, 456, 438, 366, 246, 220, 187, 165, 146; negative-ion HRFABMS *m/z* 501.3212 $[M - H]^-$, $C_{30}H_{45}O_6$ requires 501.3216.

Acetylation of 11. A mixture of **11** (8 mg) in Ac_2O (0.5 mL) and pyridine (0.5 mL) was kept standing at room temperature overnight. The mixture was concentrated to dryness, then was purified by column chromatography on Si gel with $CHCl_3$ to furnish the diacetate (7.5 mg) as colorless needles (from C_6H_6−hexane): mp 264−265 °C; ^1H NMR ($CDCl_3$) δ 0.73 (3H, s, H-24), 0.80 (3H, s, H-25), 0.95 (3H, d, J = 6.5 Hz, H-30), 0.99 (3H, s, H-26), 1.10 (3H, s, H-23), 1.21 (3H, s, H-29), 1.29 (3H, s, H-27), 2.16 (6H, s, OAc × 2), 2.52 (1H, s, H-18), 2.55 (1H, m, H-16α), 5.04 (1H, s, H-3), 5.08 (1H, s, H-1), 5.30 (1H, t, J = 3 Hz, H-12); EIMS *m/z* 586 (M^+), 480, 438, 408, 366, 146.

Isolation of Oleanolic Acid (1) and Ursolic Acid (6) from *P. glandulosa*. The dried leaves of *Pr. glandulosa* (1.3 kg) were extracted with MeOH, and the MeOH extract (225 g) was adsorbed on Celite. The Celite−MeOH mixture was washed with hexane, $CHCl_3$, Me_2CO, and MeOH, successively. The $CHCl_3$-soluble fraction (12 g) was repeatedly chromatographed on Si gel using $CHCl_3$−MeOH (1:0 → 4:1), C_6H_6−EtOAc (9:1 → 4:1), and hexane−EtOAc (8:1 → 2:1) as eluents to give oleanolic acid (**1**) (20 mg) and ursolic acid (**6**) (186 mg).

Isolation of Oleanolic Acid (1) from *Ph. juniperinum*. The dried whole plant of *Ph. juniperinum* (4.5 kg) was extracted with MeOH, and the MeOH extract (750 g) was adsorbed on Celite. The Celite−MeOH mixture was washed with *n*-hexane, $CHCl_3$, Me_2CO, and MeOH, successively. The $CHCl_3$-soluble fraction (70 g) was repeatedly chromatographed on Si gel using $CHCl_3$−MeOH (1:0 → 9:1) and C_6H_6−EtOAc (10:1 → 3:1) as eluents to afford oleanolic acid (**1**) (12.6 g).

Isolation of Oleanolic Acid (1) and Betulinic acid (15) from *T. gymnanthera*. The 95% EtOH extract (45 g) of *T. gymnanthera* (300 g) was fractioned by *n*-hexane, $CHCl_3$, and EtOAc, respectively. The $CHCl_3$-soluble fraction (6 g) was repeatedly chromatographed on Si gel using $CHCl_3$−*n*-hexane (1:1 → 4:0) as eluents to afford oleanolic acid (**1**) (106 mg) and betulinic acid (**15**) (87 mg).

General Procedure for Preparation of 3-*O*-Acyl Oleanolic Acid Derivatives (18−26). A mixture of oleanolic acid, an appropriate acid anhydride (2.5−10 equivalent mol) and (dimethylamino)pyridine (1 equivalent mol) in pyridine (5−10 mL) was refluxed overnight. The reaction mixture was diluted with ice-water and extracted with $CHCl_3$. The organic layer was washed with H_2O, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by Si gel column chromatography to afford the product. Separation of a mixture of **18** and **19** was achieved by semipreparative-scale HPLC.

Oleanolic acid 3-*O*-(3′,3′-dimethyl)succinate (18): colorless rods (from MeOH−EtOAc); mp 268−270 °C; $[\alpha]^{27}_D$ +59.2° [*c* 0.6, MeOH + $CHCl_3$ (1:1)]; ^1H NMR (pyridine-d_5, 400 MHz) δ 0.82 (3H, s, H-25), 0.93 (3H, s, H-24), 0.96 (6H, s, H-23 and H-29), 0.98 (3H, s, H-26), 1.01 (3H, s, H-30), 1.26 (3H, s, H-27), 1.54 (6H, s, dimethylsuccinyl CH_3), 2.88, 2.95 (each 1H, d, J = 15.5 Hz, H_2-2′), 3.30 (1H, dd, J = 4.0, 13.5 Hz, H-18), 4.76 (1H, dd, J = 4.5, 11.5 Hz, H-3), 5.46 (1H, t, J = 3 Hz, H-12); ^{13}C NMR (pyridine-d_5, 75 MHz) δ 15.4 (C-25), 17.1 (C-24), 17.4 (C-26), 18.5 (C-6), 23.7 (C-16), 23.7 (C-11), 23.8 (C-2), 23.9 (C-30), 25.9 (CH_3-3′), 26.1 (CH_3-3′), 26.2 (C-27), 28.2 (C-23), 28.3 (C-15), 31.0 (C-20), 33.0 (C-7), 33.2 (C-15), 33.3 (C-22), 34.3 (C-29), 37.2 (C-10),

37.9 (C-4), 38.2 (C-1), 39.7 (C-8), 40.9 (C-3′), 42.0 (C-18), 42.2 (C-14), 45.2 (C-2′), 46.5 (C-19), 46.7 (C-17), 47.9 (C-9), 55.6 (C-5), 80.9 (C-3), 122.4 (C-12), 144.8 (C-13), 171.5 (dimethylsuccinyl COO), 179.2 (dimethylsuccinyl COOH), 180.1 (C-28); positive FABMS *m/z* 607.3970 ([M + Na]$^+$), $C_{36}H_{56}NaO_6$ requires 607.3974.

Oleanolic acid 3-*O*-(2′,2′-dimethyl)succinate (19): a white crystalline powder (from MeOH−EtOAc); mp 275−278 °C; $[\alpha]^{28}_D$ +59.8° [*c* 0.7, MeOH + $CHCl_3$ (1:1)]; ^{1}H NMR (pyridine-d_5, 400 MHz) δ 0.82 (3H, s, H-25), 0.94 (3H, s, H-24), 0.97 (3H, s, H-29), 1.00 (3H, s, H-26), 1.02 (3H, s, H-30), 1.04 (3H, s, H-23), 1.27 (3H, s, H-27), 1.49 (6H, s, dimethylsuccinyl CH_3), 2.91, 2.96 (each 1H, d, J = 16.5 Hz, H_2-3′), 3.31 (1H, dd, J = 4.0, 13.5 Hz, H-18), 4.78 (1H, dd, J = 4.5, 11.5 Hz, H-3), 5.47 (1H, t, J = 3.5 Hz, H-12); ^{13}C NMR (pyridine-d_5, 75 MHz) δ 15.3 (C-25), 17.1 (C-24), 17.4 (C-26), 18.4 (C-6), 23.6 (C-16), 23.7 (C-11), 23.8 (C-2), 23.8 (C-30), 25.9 (C-2′-CH_3), 25.9 (C-2′-CH_3), 26.2 (C-27), 28.3 (C-23), 28.3 (C-15), 31 (C-20), 33.1 (C-7), 33.2 (C-22), 33.3 (C-29), 34.3 (C-21), 37.2 (C-10), 38.1 (C-4), 38.1 (C-1), 39.7 (C-8), 41 (C-2′), 42 (C-18), 42.2 (C-14), 44.8 (C-3′), 46.5 (C-19), 46.7 (C-17), 47.9 (C-9), 55.7 (C-5), 80.6 (C-3), 122.4 (C-12), 144.9 (C-13), 174 (dimethylsuccinyl COO−), 176.7 (dimethylsuccinyl COOH), 180.2 (C-28); positive FABMS *m/z* 607.3973 ([M + Na]$^+$), $C_{36}H_{56}NaO_6$ requires 607.3974.

Oleanolic acid 3-*O*-(3′,3′-dimethyl)glutarate (20): colorless needles (from MeOH), mp 244−245 °C; $[\alpha]^{22}_D$ +54.9° [*c* 0.51, MeOH + $CHCl_3$ (1:1)]; ^{1}H NMR (pyridine-d_5, 300 MHz) δ 0.84 (3H, s, H-25), 0.93 (3H, s, H-24), 0.97, 0.98 (6H, s, H-23 and H-29), 0.99 (3H, s, H-26), 1.02 (3H, s, H-30), 1.29 (3H, s, H-27), 1.37, 1.38 (each 3H, s, dimethylglutaryl CH_3), 2.78 (4H, m, dimethylglutaryl H_2-2′ and 4′), 3.30 (1H, dd, J = 4, 14 Hz, H-18), 4.74 (1H, dd, J = 5, 11.5 Hz, H-3), 5.47 (1H, t-like, H-12); ^{13}C NMR (pyridine-d_5, 75 MHz) δ 15.5 (C-25), 17.3 (C-24), 17.5 (C-26), 18.6 (C-6), 23.8 (C-16), 23.9 (C-11), 23.9 (C-30), 24.2 (C-2), 26.3 (C-27), 28.1 (dimethylglutaryl CH_3), 28.4 (C-23 and 15), 31.1 (dimethylglutaryl C-3′), 32.8 (C-20), 33.2 (C-7), 33.3 (C-22), 33.4 (C-29), 34.4 (C-21), 37.3 (C-10), 38 (C-1), 38.4 (C-4), 39.8 (C-8), 42.1 (C-18), 42.3 (C-14), 46.1 (dimethylglutaryl C-2′ and 4′), 46.6 (C-19), 46.8 (C-17), 48 (C-9), 55.7 (C-5), 80.9 (C-3), 122.5 (C-13), 145 (C-14), 172.2 (dimethylglutaryl COO), 174.7 (dimethylglutaryl COOH), 180.5 (C-28); negative HRFABMS *m/z* 597.4157 ([M − H]$^-$), $C_{37}H_{57}O_6$ requires 597.4155.

Oleanolic acid 3-*O*-diglyorate (21): colorless needles (from MeOH); mp 260−263 °C; $[\alpha]^{22}_D$ +64.4° [*c* 0.36, MeOH + $CHCl_3$ (1:1)]; ^{1}H NMR ($CDCl_3$ + CD_3OD, 400 MHz) δ 0.81 (3H, s, H-25), 0.88 (3H, s, H-24), 0.90 (3H, s, H-29), 0.91 (3H, s, H-23), 0.94 (3H, s, H-26), 0.96 (3H, s, H-30), 1.17 (3H, s, H-27), 2.85 (1H, dd, J = 4, 14 Hz, H-18), 4.19 (2H, s, H-4′), 4.25, 4.29 (each 1H, d, J = 16.5 Hz, H-2′), 4.63 (1H, dd, J = 5, 10.5 Hz, H-3), 5.28 (1H, t, J = 3.5 Hz, H-12); ^{13}C NMR ($CDCl_3$ + CD_3OD, 100 MHz) δ 15.7 (C-25), 16.9 (C-26), 17.2 (C-24), 18.7 (C-6), 23.5 (C-11), 23.8 (C-30), 23.9 (C-16), 23.9 (C-2), 26.3 (C-27), 28.2 (C-15), 28.6 (C-23), 31.1 (C-20), 33.1 (C-22), 33.2 (C-7), 33.3 (C-29), 34.3 (C-21), 37.4 (C-10), 38.3 (C-4), 38.6 (C-1), 39.8 (C-8), 41.8 (C-14), 42.3 (C-18), 46.5 (C-19), 46.9 (C-17), 48.2 (C-9), 56 (C-5), 68.2 (diglucoryl CH_2), 70.7 (diglucoryl CH_2), 84.7 (C-3), 122.5 (C-12), 144.5 (C-13), 173.2 (diglycoryl COO−), 175.5 (diglycoryl COOH), 181.2 (C-28); negative HRFABMS *m/z* 571.3637 ([M − H]$^-$), $C_{34}H_{51}O_7$ requires 571.3635.

Oleanolic acid 3-*O*-succinate (22): colorless needles from C_6H_6, mp 261−263 °C; $[\alpha]^{23}_D$ +60.0° (*c* 0.21, MeOH); ^{1}H NMR (pyridine-d_5, 500 MHz) δ 0.83 (3H, s, H-25), 0.93 (3H, s, H-24), 0.96 (3H, s, H-29), 0.98 (3H, s, H-23), 0.99 (3H, s, H-26), 1.02 (3H, s, H-30), 1.27 (3H, s, H-27), 2.89, 2.93 (each 2H, m, succinyl CH_2), 3.29 (1H, dd, J = 4, 14 Hz, H-18), 4.78 (1H, dd, J = 5, 11 Hz, H-3), 5.47 (1H, t-like, H-12); ^{13}C NMR (pyridine-d_5, 100 MHz) δ 15.3 (C-25), 16.9 (C-24), 17.3 (C-26), 18.4 (C-6), 23.6 (C-16), 23.7 (C-11 and C-30), 23.8 (C-2), 26.1 (C-27), 28.1 (C-23), 28.2 (C-15), 30.9 (C-20), 33.0 (C-22), 33.1 (C-7), 33.2 (C-29), 34.2 (C-21), 37.1 (C-10), 37.9 (C-4), 38.1 (C-1), 39.7 (C-8), 41.9 (C-18), 42.1 (C-14), 46.4 (C-19), 46.6 (C-17), 47.8 (C-9), 55.5 (C-5), 80.9 (C-3), 122.3 (C-12), 144.8 (C-13), 180.0 (C-28); succinyl moiety, 29.9, 30.3, 172.4, 174.7; negative ion HRFABMS *m/z* 555.3685 [M − H]$^-$, $C_{34}H_{51}O_6$ requires 555.3685.

Oleanolic acid 3-*O*-glutarate (23): colorless needles from $CHCl_3$, mp 222−223 °C; $[\alpha]^{24}_D$ +84.8° (*c* 0.40, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.74 (3H, s, H-25), 0.85 (3H, s, H-24), 0.88 (3H, s, H-29), 0.91 (3H, s, H-23), 0.94 (3H, s, H-26), 0.95 (3H, s, H-30), 1.13 (3H, s, H-27), 2.42 (4H, m, glutaryl CH_2), 2.82 (1H, br d, J = 14 Hz, H-18), 4.52 (1H, dd, J = 5, 11 Hz, H-3), 5.27 (1H, t-like, H-12); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 15.4 (C-25), 16.8 (C-24), 17.1 (C-26), 18.2 (C-6), 20.16, 22.8 (C-16), 23.4 (C-2), 23.5 (C-11), 23.6 (C-30), 25.9 (C-27), 28.2 (C-23 and C-15), 30.6 (C-20), 32.4 (C-22), 33.1 (C-7), 33.8 (C-29), 37.0 (C-21), 37.7 (C-10 and C-4), 37.9 (C-1), 39.3 (C-8), 40.9 (C-18), 41.5 (C-14), 45.8 (C-19), 46.5 (C-17), 47.4 (C-9), 55.2 (C-5), 81.0 (C-3), 122.6 (C-12), 143.6 (C-13), 179.4 (C-28); glutaryl moiety: 27.7, 29.7, 32.4, 172.4, 184.9; *anal.* C 73.75%, H 9.62%, calcd for $C_{35}H_{54}O_6$, C 73.65%, H 9.54%.

Oleanolic acid 3-*O*-(3′-methyl)glutarate (24): colorless needles from $CHCl_3$, mp 205−206 °C; $[\alpha]^{24}_D$ +44.9° (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.74 (3H, s, H-25), 0.85 (3H, s, H-24), 0.87 (3H, s, H-29), 0.91 (3H, s, H-23), 0.92 (3H, s, H-26), 0.94 (3H, s, H-30), 1.06 (3H, d, 3-methyl glutaryl CH_3), 1.12 (3H, s, H-27), 2.29, 2.45 (each 2H, m, 3-methyl glutaryl CH_2), 2.81 (1H, br d, J = 14 Hz, H-18), 4.53 (1H, dd, J = 5, 11 Hz, H-3), 5.27 (1H, t-like, H-12); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 15.3 (C-25), 16.8 (C-24), 17.1 (C-26), 18.2 (C-6), 22.8 (C-16), 23.4 (C-2), 23.6 (C-11 and C-30), 25.9 (C-27), 28.1 (C-23), 28.2 (C-15), 30.9 (C-20), 32.5 (C-22), 33.0 (C-7), 33.7 (C-29), 37.0 (C-21), 37.7 (C-10), 37.9 (C-4), 37.9 (C-1), 39.2 (C-8), 40.8 (C-18), 41.5 (C-14), 45.8 (C-19), 46.6 (C-17), 47.5 (C-9), 55.2 (C-5), 81.0 (C-3), 122.5 (C-12), 143.5 (C-13), 178.8 (C-28); succinyl moiety, 19.9, 27.3, 40.5, 51.53, 171.9, 184.8; *anal.* C 72.18%, H 9.28%, calcd for $C_{36}H_{56}O_6 \cdot 3/4H_2O$, C 72.27%, H 9.68%.

Oleanolic acid 3-*O*-(3′-methyl-3′-ethyl)glutarate (25): colorless needles from $CHCl_3$, mp 266−267 °C; $[\alpha]^{24}_D$ +58.7° (*c* 0.42, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.73 (3H, s, H-25), 0.82 (3H, s, H-24), 0.88 (3H, s, H-29), 0.91 (3H, s, H-23), 0.94 (3H, s, H-26), 0.97 (3H, s, H-30), 1.02, 1.09 (1.5 H each, s, (*RS*)-3′-methyl-3′-ethyl-glutaryl CH_3), 1.13 (3H, s, H-27), 2.43−2.75 (4H, m, (*RS*)-3′-methyl-3′-ethyl-glutaryl $COCH_2$), 2.81 (1H, dd, J = 2, 14 Hz, H-18), 4.50, 4.53 (0.5 H each, both t, J = 11 Hz, H-3), 5.28 (1H, t-like, H-12); ^{13}C NMR

($CDCl_3$, 75 MHz) δ 15.5 (C-25), 17.0 (C-24), 17.5 (C-26), 18.3 (C-6), 22.6 (C-16), 23.3 (C-2), 23.6 (C-11 and C-30), 25.9 (C-27), 27.7 (C-23), 28.3 (C-15), 30.6 (C-20), 32.3 (C-22), 33.0 (C-7), 33.7 (C-29), 37.0 (C-21), 37.6, 37.6 (C-10 and C-4), 37.7 (C-1), 39.2 (C-8), 40.8 (C-18), 41.3 (C-14), 45.8 (C-19), 46.5 (C-17), 47.3 (C-9), 55.1 (C-5), 80.8, 81.0 (0.5 C each, C-3), 122.6 (C-12), 143.7 (C-13), 178.2 (C-28); (*RS*)-3′-methyl-3′-ethyl-glutaryl moiety: δ 7.9, 8.0, 24.4, 24.6, 32.5, 32.9, 33.4, 35.2, 41.8, 42.3, 42.7, 43.1, 171.9, 172.0 (each 0.5C), 184.8; *anal.* C 73.84%, H 9.75%, calcd for $C_{38}H_{60}O_6 \cdot 1/4H_2O$, C 73.93%, H 9.88%.

Oleanolic acid 3-*O*-(3′,3′-tetramethylene)glutarate (26): colorless needles from $CHCl_3$, mp 259−260 °C; $[\alpha]^{24}_D$ +44.1° (*c* 0.20, $CHCl_3$); ^1H NMR ($CDCl_3$ + CD_3OD 1:1, 300 MHz) δ 0.61 (3H, s, H-25), 0.69 (3H, s, H-24), 0.70 (3H, s, H-29), 0.72 (3H, s, H-23), 0.75 (3H, s, H-26), 0.76 (3H, s, H-30), 0.97 (3H, s, H-27), 1.44−1.49 (8H, m, 4 H_2 of suberoyl), 2.34, 2.38 (each 2H, m, $COCH_2$ of suberoyl), 2.50 (1H, dd, J = 4, 14 Hz, H-18), 4.31 (1H, t, J = 8.5 Hz, H-3), 5.09 (1H, t-like, H-12); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 15.0 (C-25), 16.4 (C-24), 16.5 (C-26), 18.0 (C-6), 22.8 (C-16), 23.3 (C-2), 23.7 (C-11 and C-30), 25.6 (C-27), 27.4 (C-23), 27.8 (C-15), 30.4 (C-20), 32.4 (C-22), 32.7 (C-7), 33.6 (C-29), 37.3 (C-21), 37.6 (C-10), 37.8 (C-4), 37.9 (C-1), 39.1 (C-8), 41.0 (C-18), 41.8 (C-14), 45.7 (C-19), 46.2 (C-17), 47.3 (C-9), 55.2 (C-5), 81.1 (C-3), 122.0 (C-12), 143.7 (C-13), 174.6 (C-28); suberoyl moiety: δ 23.2, 23.8, 32.3, 36.7, 41.5, 41.9, 172.7, 180.7; *anal.* C 72.65%, H 9.45%, calcd for $C_{39}H_{60}O_6 \cdot H_2O$, C 72.86%, H 9.72%.

Oxidation of Oleanolic Acid (27). A mixture of oleanolic acid (**1**) (180 mg) and PCC (170 mg) in CH_2Cl_2 (70 mL) was stirred at room temperature for 25 h. The solid was filtered using Celite and discarded, and the filtrate was evaporated. The reaction product was purified by Si gel column chromatography using $CHCl_3$ as eluent to give 3-oxooleanolic acid (**27**) (102 mg) as colorless prisms (from *n*-hexane−EtOAc), mp 201−203 °C; $[\alpha]^{25}_D$ +88.1° (*c* 0.49, MeOH); HREIMS *m/z* 453.3372 $[M - H]^+$, $C_{30}H_{45}O_3$ requires 453.3368.

Preparation of Potassium Salts of Oleanolic Acid (28). A solution of oleanolic acid (105 mg) was treated with 2% KOH in Me_2CO-H_2O (1:1) at room temperature for 30 min. After removal of Me_2CO by evaporation, the resulting aqueous solution was subjected to pass over a MCI-gel CHP20P column, and washed with H_2O to remove excess amounts of KOH. Subsequent elution with MeOH gave potassium oleanate (**27**) (98 mg) as a white powder; mp 285−288 °C (decomp); FABMS *m/z* 495 $[M + H]^+$, 533 $[M + K]^+$.

Anti-HIV Assay. The T cell line, H9, was maintained in continuous culture with complete medium (RPMI 1640 with 10% fetal calf serum [FCS] supplemented with L-glutamine) at 5% CO_2 and 37 °C. Aliquots of this cell line were used in experiments only when in log-phase of growth.

Test samples were first dissolved in dimethyl sulfoxide. The following are the final drug concentrations routinely used for screening: 100, 20, 4, and 0.8 μg/mL. For active agents, additional dilutions are prepared for subsequent testing so that an accurate EC_{50} values (see definition below) could be achieved.

As the test samples were being prepared, an aliquot of H9 cells was infected with HIV-1 (IIIB isolate), while another aliquot was mock-infected with complete medium. The mock-infected sample was used for toxicity determinations (IC_{50}, see definition below). The stock virus used for these studies typically had a $TCID_{50}$ value of 10^4 Infectious Units (IU)/mL. The appropriate amount of virus for a multiplicity of infection between 0.1 and 0.01 IU/cell was added to the first aliquot of cells. The other aliquot of cells received only culture medium and was then incubated under identical conditions to the HIV-infected cells. After a 4-h incubation at 37 °C and 5% CO_2, both cell populations were washed three times with fresh medium and then added to the appropriate wells of a 24-well plate containing the various concentrations of the test drug or culture medium (positive infected control/negative-control drug). In addition, AZT was also assayed during each experiment as a positive-control drug. The plates were incubated at 37 °C and 5% CO_2 for 4 days. Cell-free supernatants were collected on day 4 and tested by an in-house p24 antigen ELISA assay; p24 antigen is a core protein of HIV and, therefore, is an indirect measure of virus present in the supernatants. Toxicity was determined by performing cell counts by a coulter counter on the mock-infected cells, which had either received culture medium (no toxicity) or test sample or AZT. If a test sample had suppressive capability and was not toxic, its effects are reported in the following terms: IC_{50}, the concentration of test sample that was toxic to 50% of the mock-infected cells; EC_{50}, the concentration of the test sample that was able to suppress HIV replication by 50%; and therapeutic index (TI), the ratio of IC_{50} to EC_{50}.

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