Anti-AIDS Agents. 48.[1] Anti-HIV Activity of Moronic Acid Derivatives and the New Melliferone-Related Triterpenoid Isolated from Brazilian Propolis

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A new triterpenoid named melliferone (**1**), three known triterpenoids, moronic acid (**2**), anwuweizonic acid (**3**), and betulonic acid (**4**), and four known aromatic compounds (**5**−**8**) were isolated from Brazilian propolis and tested for anti-HIV activity in H9 lymphocytes. Moronic acid (**2**) showed significant anti-HIV activity (EC_{50} <0.1 μg/mL, TI >186) and was modified to develop more potent anti-AIDS agents.

In a continuing effort to identify new anti-AIDS agents with novel mechanisms, we have coupled the discovery of anti-HIV principles from biologically active natural resources with modification of these lead compounds to develop more potent anti-AIDS analogues. Propolis is a resinous material collected by honeybees from parts of plants, buds, and exudates and has been used as a folk medicine since ca. 300 BC.[2] Recently, numerous biological properties have been reported including cytotoxic,[3] antiherpes,[4] antitumor,[5,6] radical scavenging,[7] and antimicrobial[8] activities. Accordingly, in our continuous screening of natural products for anti-AIDS agents, we tested several Brazilian propolis samples, which belong to different classifications based on morphological and phytochemical characteristics proposed by Park et al.[9] A methanolic extract of a Group I Brazilian propolis demonstrated significant anti-HIV activity (EC_{50} <0.10 μg/mL, TI >171). The new triterpenoid melliferone (**1**, 3-oxoolean-11-en-13β,28-olide), three known triterpenoids, moronic acid (**2**), anwuweizonic acid (**3**), and betulonic acid (**4**), and four aromatic compounds (**5**−**8**) were isolated from this propolis (Figure 1). Moronic acid (**2**) showed potent anti-HIV activity (EC_{50} <0.1 μg/mL, TI >186). Therefore, the lead compound was modified to enhance anti-HIV activity and develop more potent anti-AIDS agents.

Results and Discussion

Isolation of Triterpenoids and Aromatic Compounds. In our screening of natural products, a methanolic extract of a Brazilian propolis collected in southern Brazil near the Uruguayan border showed significant anti-HIV activity (EC_{50} <0.10 μg/mL, TI >171). *Myrceugenia euosma* (O. Berg) Legrand (Myrtaceae) is an abundant cultivar in this region and has been identified as the major source of this particular propolis.[10−12] The propolis extract was fractionated to afford hexane, chloroform, ethyl acetate, and methanol fractions. From TLC analysis, the chloroform fraction was abundant in triterpenoids. Because several triterpenoids have been reported to show anti-HIV activity,[13,14] we chromatographed the chloroform fraction on Si gel and isolated a new triterpenoid, melliferone (**1**), together with seven known compounds, moronic acid (**2**), anwuweizonic acid (**3**), betulonic acid (**4**), and four aromatic compounds (**5**−**8**). The three known triterpenoids (**2**−**4**) and four aromatic compounds (**5**−**8**) were identified by comparing their NMR spectral data to the literature or ^{1}H NMR values of an authentic sample.[15−21]

Structure of Melliferone (1). Melliferone (**1**) was obtained from the chloroform-eluting fraction of the methanol extract of propolis by repeated silica gel chromatography. It showed a quasimolecular ion peak at *m*/*z* 451.3195 $[M - H]^-$ (*m*/*z* 451.3212 calcd for $C_{30}H_{43}O_3$) in the high-resolution FABMS spectrum. The ^{13}C NMR spectrum of **1** exhibited signals for 30 carbons and was similar to that of 3β-benzoyloxyoleana-11-en-13β,28-olide isolated from *Ploiarium alternifolium*, except for the presence of a 3-ketone group (δ_C 216.8 (s)) as well as the absence of a 3β-benzoyloxy group.[22] In addition, except for the absence of the secondary hydroxy group at C-3, the ^{1}H NMR spectral data of **1** resembled those of 3β-hydroxyolean-11-en-13β,28-olide, which was obtained from *Hyptis albida*.[23] From this spectral evidence, we propose the structure of this new triterpenoid, melliferone, to be represented as **1** (3-oxoolean-11-en-13β,28-olide).

Anti-HIV Activity of Natural Products. The anti-HIV data of compounds isolated from a Brazilian propolis are shown in Table 1. Moronic acid (**2**), which was isolated in large quantities (1.5 g), showed significant anti-HIV activity (EC_{50} <0.1 μg/mL, TI >186). In contrast, anwuweizonic acid (**3**) did not suppress HIV replication in this assay, and betulonic acid (**4**), which exhibited considerable anti-HIV activity in a previous study,[13] showed weak suppression. In addition, the aromatic constituents (**5**−**8**) did not show any effective anti-HIV activity, although **7** showed some suppression of the proliferation of HIV-infected T cells. Thus, moronic acid (**2**) is the major anti-HIV principle in this Brazilian propolis.

Moronic Acid Derivatives of Anti-AIDS Activity. Our group has previously reported the modification of betulinic acid-related and oleanolic acid-related triterpenoids to develop more potent anti-AIDS agents.[13,14] Esterification of the 3β-hydroxy groups of these two triterpenoids has resulted in enhanced anti-HIV activity. Accordingly, as shown in Scheme 1, we made a similar modification of moronic acid (**2**) to produce first the 3β-alcohol (**9**) and

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Table 1. Anti-HIV Activities of Compounds Isolated from Brazilian Propolis and Moronic Acid Derivatives

compound	IC_{50} (μg/mL)[a]	EC_{50} (μg/mL)[b]	TI[c]
1	0.205	no suppression	no suppression
2	18.6	<0.1	>186
3	2.14	no suppression	no suppression
4	1.8[d]	0.22[d]	8[d,e]
5	18.8	no suppression	no suppression
6	>100	no suppression	no suppression
7	18.9	2.19	8.62[e]
8	2.07	no suppression	no suppression
9	22.1	2.70	8.17[e]
10	18.1	1.0	18.0
AZT	500	0.002 89	173 000

[a] Concentration of agent that is cytotoxic to 50% of the H9 cells. [b]Concentration of agent that inhibits viral replication in the H9 cells by 50%. [c]In vitro TI (therapeutic index), ratio of IC_{50}:EC_{50}. [d]Data taken from ref 10. [e]TI is <10; therefore, agent is not considered to be suppressive.

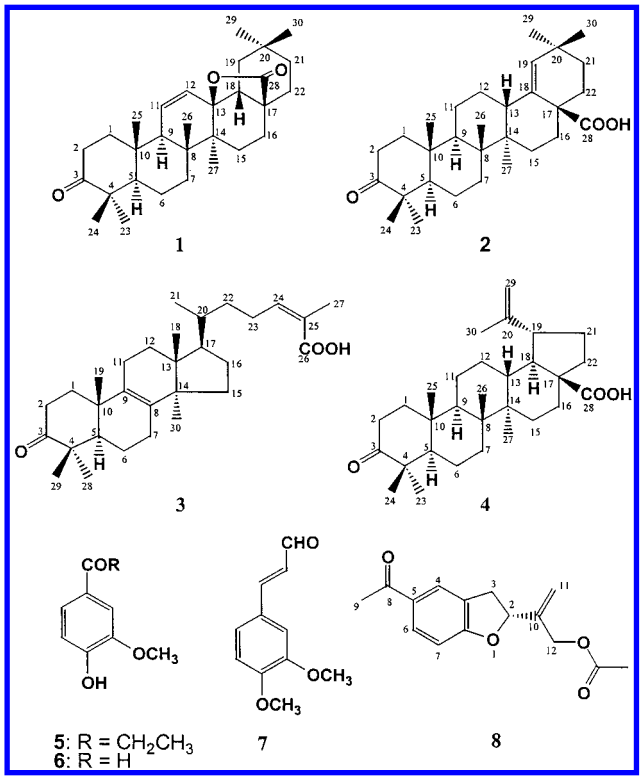


Figure 1. Compounds isolated from Brazilian propolis.

subsequently the 3β-(3′,3′-dimethylsuccinyl) ester (**10**). However, anti-HIV activity of both compounds decreased relative to that of the parent acid. In particular, the ester **10** showed a 10-fold drop in potency (EC_{50} 1.0 μg/mL, TI 18.1). We are continuing our structure−activity relationship study to determine appropriate modifications of moronic acid (**2**) and to isolate other minor triterpenoids from this Brazilian propolis.

Experimental Section

General Experimental Procedures. Optical rotations were determined on a JASCO DIP-1000 digital porarimeter (cell length 10 mm, unless otherwise indicated). The ^{1}H and ^{13}C NMR spectra were taken on a Varian Gemini 2000 300 MHz NMR spectrometer with TMS as internal reference. The EIMS spectra were measured with a VG-70E double-focus high-resolution GC mass spectrometer. The FABMS spectra were measured with a JEOL JMS-DX300.

Plant Material. A voucher specimen of *M. euosma* is deposited at the Institute of Agriculture, Campinas, Brazil.

Isolation of Melliferone (1), Moronic Acid (2), Anwuweizonic Acid (3), and Betulonic Acid (4) from Brazilian Propolis. The propolis collected by Africanized *Apis mellifera* in southern Brazil (City of Bage near Uruguay) in February 1999 was extracted with methanol to yield an extract (600 g). The extract was adsorbed on Celite and washed with hexane, chloroform, ethyl acetate, and methanol to give hexane (59 g), chloroform (293 g), ethyl acetate (1.8 g), and methanol (6.2 g) fractions, respectively. A part of the chloroform-eluting fraction (100 g) was subjected to silica gel column chromatography (CC) using a gradient solvent system (hexane−chloroform to methanol) to give 14 fractions (fractions 1 to 14). Fraction 4 was subjected to silica gel CC using a gradient solvent system (hexane−chloroform−methanol) to give 14 fractions (fractions 4-1 to -12). Fraction 4-8 was recrystallized from chloroform−hexane to give moronic acid (**2**) (1.5 g) as a powder.

Fraction 4-3 was subjected to silica gel CC using a gradient solvent system (hexanes−ethyl acetate and ethyl acetate) to give 11 fractions (fractions 4-3-1 to -11). Fraction 4-3-11 was purified by preparative thin-layer chromatography (PTLC) (hexanes−ethyl acetate (5:1)) to give betulonic acid (**4**) (22 mg).

Fraction 4-4 was subjected to silica gel CC using a gradient solvent system (hexanes−ethyl acetate, ethyl acetate, and acetone) to give 9 fractions (fractions 4-4-1 to -9). Fraction 4-4-6 was applied to PTLC (hexanes−ethyl acetate (5:1)) to give 3 fractions (fractions 4-4-6-1 to -3). The first fraction contained anwuweizonic acid (**3**) (161 mg), and the third fraction contained 4-hydroxy-3-methoxypropiophenone (**5**) (13 mg).

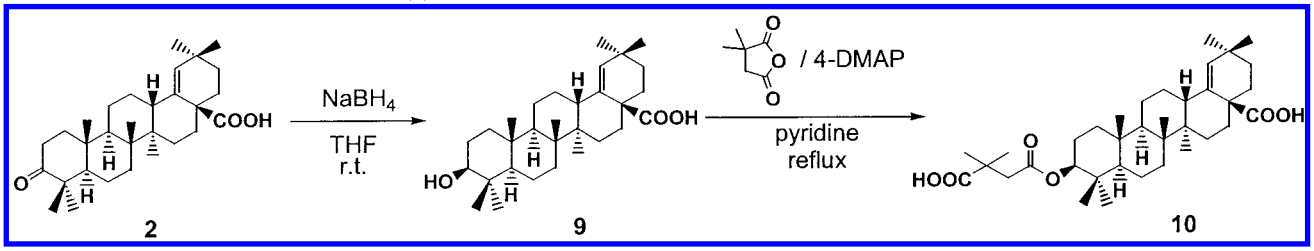
Fraction 4-4-7 was subjected to aluminum oxide (Grade III) CC using a gradient solvent system (hexanes−ethyl acetate (5% to 50%) and ethyl acetate) to give 4-hydroxy-3-methoxybenzaldehyde (**6**) (6 mg).

Fraction 4-5 was subjected to silica gel CC using a gradient solvent system (hexanes−ethyl acetate) to give 7 fractions (fractions 4-5-1 to -7). Fraction 4-5-3 was subjected to silica gel CC (chloroform−methanol (50:1)) to give 5 fractions (fractions 4-5-3-1 to -5). Fraction 4-5-3-2 was applied to PTLC (chloroform−methanol (20:1)) to give 3 fractions (fractions 4-5-3-2-1 to -3). The first fraction (fraction 4-5-3-2-1) was applied to PTLC (ethyl acetate−toluene−acetic acid (5:25:1)) to give melliferone (**1**) (5 mg).

Fraction 4-5-4 was applied to PTLC (chloroform−acetone (20:1)) to give 12-acetoxytremetone (**8**) (6 mg), and fraction 4-5-5 was treated similarly to give 3-(3,4-dimethoxyphenyl)-2-propenal (**7**).

Melliferone (1): colorless amorphous solid; $[\alpha]_D$ +54.0° (*c* 0.24, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.44 (1H, dd, J = 10.2, 3.3 Hz, H-11), 6.01 (1H, dd, J = 10.2, 1.6 Hz, H-12), 0.86, 0.95, 1.03 × 2, 1.05, 1.07, 1.08 (each 3H, s, H-23, H-24, H-25, H-26, H-27, H-29, H-30); ^{13}C NMR ($CDCl_3$) δ 39.0 (t, C-1), 34.3 (t, C-2), 216.8 (s, C-3), 47.6 (s, C-4), 54.6 (d, C-5), 18.8 (t, C-6),

Scheme 1. Modification of Moronic Acid (**2**)



33.8 (t, C-7), 41.4 (s, C-8), 52.5 (d, C-9), 36.1 (s, C-10), 135.2 (d, C-11), 127.4 (d, C-12), 89.5 (s, C-13), 41.5 (s, C-14), 27.1 (t, C-15), 21.3 (t, C-16), 44.0 (s, C-17), 50.5 (d, C-18), 37.3 (t, C-19), 31.4 (s, C-20), 30.4 (t, C-21), 25.4 (t, C-22), 26.0 (q, C-23), 20.8 (q, C-24), 17.3 (q, C-25), 18.6 (q, C-26), 18.1 (q, C-27), 179.9 (s, C-28), 33.3 (q, C-29), 23.5 (q, C-30); EIMS *m/z* 452 (M^+); HRFABMS *m/z* 451.3195 [M − H]$^-$ (*m/z* 451.3212 calcd for $C_{30}H_{43}O_3$).

Moronic acid (2): colorless powder; $[\alpha]_D$ +59.3° (*c* 1.01, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.10 (1H, s, H-19), 1.02 (3H, s, H-23), 0.964 (3H, s, H-24), 0.89 (3H, s, H-25), 0.955 (3H, s, H-26), 0.73 (3H, s, H-27), 0.94 (3H, s, H-29), 0.91 (3H, s, H-30); ^{13}C NMR ($CDCl_3$) δ 39.7 (t, C-1), 33.9 (t, C-2), 218.3 (s, C-3), 47.1 (s, C-4), 54.7 (d, C-5), 19.5 (t, C-6), 33.4 (t, C-7), 40.4 (s, C-8), 50.3 (d, C-9), 36.8 (s, C-10), 21.4 (t, C-11), 25.9 (t, C-12), 41.3 (d, C-13), 42.4 (s, C-14), 29.2 (t, C-15), 33.6 (t, C-16), 47.8 (s, C-17), 136.4 (s, C-18), 133.0 (d, C-19), 31.9 (s, C-20), 33.3 (t, C-21), 33.2 (t, C-22), 26.7 (q, C-23), 20.8 (q, C-24), 16.4 (q, C-25), 15.7 (q, C-26), 14.7 (q, C-27), 182.8 (s, C-28), 30.2 (q, C-29), 29.0 (q, C-30); EIMS *m/z* 454 (M^+).[15]

Anwuweizonic acid (3): yellow oil; $[\alpha]_D$ −7.3° (*c* 2.95, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.76 (3H, s, H-18), 1.05 (3H, s, H-19), 0.93 (3H, d, J = 6.0 Hz, H-21), 6.09 (1H, t, J = 7.5 Hz, H-24), 1.92 (3H, s, H-27), 0.89 (3H, s, H-28), 1.06 (3H, s, H-29), 1.10 (3H, s, H-30); ^{13}C NMR ($CDCl_3$) δ 35.5 (t, C-1), 34.5 (t, C-2), 218.2 (s, C-3), 47.3 (s, C-4), 51.4 (d, C-5), 20.2 (t, C-6), 26.8 (t, C-7), 132.7 (s, C-8), 134.7 (s, C-9), 37.1 (s, C-10), 21.3 (t, C-11), 30.7 (t, C-12), 44.1 (s, C-13), 50.0 (s, C-14), 29.8 (t, C-15), 28.0 (t, C-16), 50.0 (d, C-17), 15.5 (q, C-18), 19.7 (q, C-19), 36.4 (d, C-20), 18.5 (q, C-21), 35.8 (t, C-22), 27.4 (t, C-23), 147.3 (d, C-24), 125.8 (s, C-25), 173.3 (s, C-26), 20.5 (q, C-27), 24.2 (q, C-28), 26.7 (q, C-29), 21.2 (q, C-30); EIMS *m/z* 454 (M^+).[16,17]

Betulonic acid (4): colorless amorphous solid; $[\alpha]_D$ +40.1° (*c* 0.86, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.93, 0.98, 0.99, 1.02, 1.07 (each 3H, s, H-23, H-24, H-25, H-26, H-27), 4.62, 4.75 (each 1H, brs, H-29), 1.70 (3H, s, H-30); ^{13}C NMR ($CDCl_3$) δ 39.6 (t, C-1), 34.1 (t, C-2), 218.2 (s, C-3), 47.3 (s, C-4), 54.9 (d, C-5), 19.6 (t, C-6), 33.6 (t, C-7), 40.6 (s, C-8), 49.8 (d, C-9), 36.9 (s, C-10), 21.4 (t, C-11), 25.5 (t, C-12), 38.5 (d, C-13), 42.5 (s, C-14), 30.5 (t, C-15), 32.1 (t, C-16), 56.3 (s, C-17), 49.2 (d, C-18), 46.9 (d, C-19), 150.3 (s, C-20), 29.7 (t, C-21), 37.0 (t, C-22), 26.6 (q, C-23), 21.0 (q, C-24), 15.9 (q, C-25), 15.8 (q, C-26), 14.6 (q, C-27), 181.7 (s, C-28), 109.8 (t, C-29), 19.3 (q, C-30); EIMS *m/z* 454 (M^+).[18]

4-Hydroxy-3-methoxypropiophenone (5): yellow amorphous solid; ^{1}H NMR ($CDCl_3$) δ 7.53 (1H, d, J = 1.9 Hz, H-2), 6.92 (1H, d, J = 8.8 Hz, H-5), 7.52 (1H, dd, J = 8.8, 1.9 Hz, H-6), 2.93 (2H, q, J = 7.4 Hz, H-2′), 1.19 (3H, t, J = 7.4 Hz, H-3′), 3.93 (3H, s, CH_3O-3), 6.08 (1H, s, HO-4); ^{13}C NMR ($CDCl_3$) δ 129.9 (s, C-1), 113.7 (d, C-2), 146.6 (s, C-3), 150.1 (s, C-4), 109.8 (d, C-5), 123.2 (d, C-6), 199.6 (s, C-1′), 32.3 (t, C-2′), 8.6 (q, C-3′), 56.0 (q, CH_3O-3); EIMS *m/z* 180 (M^+).[19]

4-Hydroxy-3-methoxybenzaldehyde (6): colorless amorphous solid; ^{1}H NMR ($CDCl_3$) δ 7.40 (1H, d, J = 1.6 Hz, H-2), 7.02 (1H, d, J = 8.5 Hz, H-5), 7.41 (1H, dd, J = 8.5, 1.6 Hz, H-6), 9.81 (1H, s, CHO-1), 3.95 (3H, s, CH_3O-3), 6.19 (1H, s, HO-4); ^{13}C NMR ($CDCl_3$) δ 129.9 (s, C-1), 114.4 (d, C-2), 147.1 (s, C-3), 151.6 (s, C-4), 108.7 (d, C-5), 127.5 (d, C-6), 190.9 (d, CHO-1), 56.1 (q, CH_3O-3); EIMS *m/z* 152 (M^+). This compound was identified by comparison to the ^{1}H NMR spectrum of an authentic sample.

3-(3,4-Dimethoxyphenyl)-2-propenal (7): yellow amorphous solid; ^{1}H NMR ($CDCl_3$) δ 7.06 (1H, d, J = 1.9 Hz, H-2), 6.89 (1H, d, J = 8.2 Hz, H-5), 7.14 (1H, dd, J = 8.2, 1.9 Hz, H-6), 7.40 (1H, d, J = 15.7 Hz, H-1′), 6.59 (1H, dd, J = 15.7, 7.7 Hz, H-2′), 9.64 (1H, d, J = 7.7 Hz, H-3′), 3.91 (3H, s, CH_3O-3)[a], 3.90 (3H, s, CH_3O-4)[a]; ^{13}C NMR ($CDCl_3$) δ 127.0 (s, C-1), 111.1 (d, C-2), 149.4 (s, C-3), 152.0 (s, C-4), 109.8 (d, C-5), 126.7 (d, C-6), 152.8 (d, C-1′), 123.4 (d, C-2′), 193.5 (d, C-3′), 56.0 (q, CH_3O-3)[b], 55.9 (q, CH_3O-4)[b]; EIMS *m/z* 192 (M^+).[20] [a,b]Assignments may be reversed.

12-Acetoxytremetone (8): yellow oil; $[\alpha]_D$ −19.4° (*c* 0.41, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.36 (1H, t, J = 8.8 Hz, H-2), 3.16, 3.43 (each 1H, dd, J = 8.8, 15.7 Hz, H-3), 7.81 (1H, d, J = 1.9 Hz, H-4), 7.79 (1H, dd, J = 8.8, 1.9 Hz, H-6), 6.81 (1H, d, J = 8.8 Hz, H-7), 2.52 (3H, s, H-9), 5.26, 5.33 (each 1H, brs, H-11), 4.63, 4.70 (each 1H, d, J = 13.2 Hz, H-12), 2.01 (3H, s, C*H*$_3$-OCO-12); ^{13}C NMR ($CDCl_3$) δ 84.1 (d, C-2), 34.5 (t, C-3), 127.0 (s, C-3a), 130.5 (d, C-4), 130.8 (s, C-5), 125.5 (d, C-6), 109.0 (d, C-7), 163.5 (s, C-7a), 196.6 (s, C-8), 26.4 (q, C-9), 142.4 (s, C-10), 114.9 (t, C-11), 63.6 (t, C-12), 170.5 (s, CH_3OCO-12), 20.8 (q, *C*H_3OCO-12); EIMS *m/z* 260 (M^+).[21]

3*β*-Hydroxy-3-deoxymoronic Acid (9). To a stirred solution of moronic acid (**2**) (29.7 mg, 65.4 μmol) in anhydrous tetrahydrofuran (THF) was added sodium borohydride (5.5 mg, 145.4 μmol) at room temperature. After 4 h, the mixture was neutralized with 2% hydrochloric acid, diluted with brine, and extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate and, after filtration, concentrated under reduced pressure to give the target alcohol (**9**) (28.3 mg, yield 94.9%) as a colorless amorphous solid: $[\alpha]_D$ +39.8° (*c* 0.13, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 3.19 (1H, dd, J = 5.5, 11.0 Hz, H-3), 5.13 (1H, s, H-19), 0.75 (3H, s, H-23)[a], 0.95 (3H, s, H-24)[a], 0.84 (3H, s, H-25), 0.95 (3H, s, H-26), 0.74 (3H, s, H-27), 0.97 (3H, s, H-29), 0.96 (3H, s, H-30); ^{13}C NMR ($CDCl_3$) δ 38.94 (t, C-1), 27.4 (t, C-2), 79.0 (d, C-3), 38.87 (s, C-4), 55.5 (d, C-5), 18.2 (t, C-6), 34.6 (t, C-7), 40.7 (s, C-8), 51.2 (d, C-9), 37.2 (s, C-10), 20.9 (t, C-11), 26.0 (t, C-12), 41.3 (d, C-13), 42.6 (s, C-14), 29.4 (t, C-15), 33.53 (t, C-16)[b], 48.0 (s, C-17), 136.8 (s, C-18), 133.0 (d, C-19), 32.0 (s, C-20), 33.46 (t, C-21)[b], 33.37 (t, C-22)[b], 27.9 (q, C-23), 16.6 (q, C-24), 15.4 (q, C-25), 16.0 (q, C-26), 14.9 (q, C-27), 181.9 (s, C-28), 30.3 (q, C-29), 29.1 (q, C-30); EIMS *m/z* 456 (M^+).[24] [a,b]Assignments may be reversed.

3-*O*-(3′,3′-Dimethylsuccinyl)moronic Acid (10). To a mixture of **9** (21.7 mg, 47.6 μmol) and pyridine was added 4-(dimethylamino)pyridine (5.8 mg, 47.6 μmol) and 2,2-dimethylsuccinic anhydride (18.2 mg, 147.8 μmol) at room temperature and stirred under reflux. An additional 18.2 mg of 2,2-dimethylsuccinic anhydride (totally 72.8 mg, 568.2 μmol) was added to the reaction mixture each day for 5 days. Then the mixture was diluted with water and extracted with chloroform. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and after filtration, concentrated under reduced pressure. The resulting residue was subjected to silica gel CC using a gradient solvent system (chloroform−acetone (20:1 to 4:1)) to give 2 fractions. The first fraction was subjected to PTLC (chloroform−acetone (20:1)) to give 3-*O*-(3′,3′-dimethylsuccinyl)moronic acid (**10**) (16.8 mg, yield 60.5%) as a colorless amorphous solid: $[\alpha]_D$ +24.6° (*c* 1.28, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 4.50 (1H, dd, J = 5.2, 10.4 Hz, H-3), 5.13 (1H, s, H-19), 0.78 (3H, s, H-23)[a], 0.82 (3H, s, H-24)[a], 0.85 (3H, s, H-25), 0.95 (3H, s, H-26), 0.72 (3H, s, H-27), 0.98 (3H, s, H-29), 0.97 (3H, s, H-30), 2.74, 2.50 (each 1H, d, J = 15.7 Hz, H-2′), 1.25, 1.28 (each 3H, s, CH_3-3′); ^{13}C NMR ($CDCl_3$) δ 38.3 (t, C-1), 23.6 (t, C-2), 81.5 (d, C-3), 37.8 (s, C-4), 55.3 (d, C-5), 18.2 (t, C-6), 34.2 (t, C-7), 40.6 (s, C-8), 50.6 (d, C-9), 37.1 (s, C-10), 21.0 (t, C-11), 25.7 (t, C-12), 41.2 (d, C-13), 42.5 (s, C-14), 29.4 (t, C-15), 33.5 (t, C-16)[b], 48.1 (s, C-17), 136.6 (s, C-18), 133.1 (d, C-19), 32.0 (s, C-20), 33.4 (t, C-21)[b], 33.3 (t, C-22)[b], 28.2 (q, C-23), 16.9 (q, C-24), 16.6 (q, C-25), 16.7 (q, C-26), 14.7 (q, C-27), 182.9 (s, C-28), 30.3 (q, C-29), 29.0 (q, C-30), 170.8 (s, C-1′), 45.1 (t, C-2′), 40.5 (s, C-3′), 183.1 (s, C-4′), 24.7, 26.1 (q, CH_3-3′); EIMS *m/z* 540 (M − COOH)$^+$; HR-FABMS *m/z* 583.4003 [M − H]$^-$ (*m/z* 583.3999 calcd. for $C_{36}H_{55}O_6$). [a,b]Assignments may be reversed.

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 (DMSO). The following were the final drug concentrations routinely used for screening: 100, 20, 4, and 0.8 μ/mL, but for active agents, additional dilutions were prepared for subsequent testing so that an accurate EC_{50} value could be achieved. As the test samples were being prepared, an aliquot of the T cell line, H9, was infected with HIV-1 (IIIB isolate) while another aliquot was mock-infected with complete medium. The mock-infected was used for toxicity determinations (IC_{50}). The stock virus used for these studies typically had a $TCID_{50}$ value of 10^4

infectious units/mL. The appropriate amount of virus for a multiplicity of infection (moi) between 0.1 and 0.01 infection units/cell was added to the first aliquot of H9 cells. The other aliquot of H9 cells received only culture medium and then was incubated under identical conditions as the HIV-infected H9 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 drug control). In addition, AZT was also assayed during each experiment as a positive drug control. The plates were incubated at 37 °C and 5% CO_2 for 4 days. Cell-free supernatants were collected on day 4 for use in our 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 H9 cells that had received either culture medium (no toxicity) or test sample or AZT.

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