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A REINVESTIGATION OF *MAPROUNEA* TRITERPENES[1]

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ABSTRACT.—Anti-HIV activity and the inhibition of phorbol ester receptor binding activity in two species of *Maprounea* were traced to small amounts of highly potent phorbol esters of the daphnane type. The triterpenes previously isolated from this genus were found to be devoid of biological activity when scrupulously purified. Four new triterpene esters were elucidated; two [**3**,**4**] were found in *M. africana*, while three [**4**,**6**,**7**] were found in *M. membranacea*. Nmr assignments have also been made for two previously known compounds [**2**,**5**] in this group.

In the course of investigating the HIV-inhibitory and phorbol ester receptor-binding activities of plants in the family Euphorbiaceae (2), we detected both activities in extracts of *Maprounea africana* Muell.-Arg. collected in the Central African Republic. This plant has an interesting history of medicinal use in central and eastern Africa, the roots having been used as a purgative and cure for syphilis (3,4), in witchcraft, and as a facial lotion for ailments of the eye (4,5), with the sap having been used to dress the penis after circumcision (5).

A series of cytotoxic triterpenes had been reported from this species (6); the structures of the compounds were subsequently revised on the basis of X-ray crystallographic and nmr evidence (7,8). In addition, the structure of compound **1** was recently revised again (9). When we attempted to reisolate these triterpenes for detailed biological evaluation, it became clear that the reported triterpenes were, in fact, contaminated with small amounts of potent phorbol esters, which were responsible for the anti-HIV and phorbol ester receptor binding activity. Examination of the related species *M. membranacea* Pax & K. Hoffman revealed similar, though less potent, activity in our biological assays. In addition, four novel triterpene esters were isolated from the two species and their structures determined.

RESULTS AND DISCUSSION

The crude extracts of both species of *Maprounea* displayed significant activity in the primary anti-HIV screen (10) as well as displacement of [^{3}H]-phorbol dibutyrate (PDBu) from rat brain membranes (2). An NCI repository sample of compound **1** was also active in both assays. Analytical isocratic hplc of the repository samples of **1** and **5** suggested that they were of reasonable (>95%) purity. On-line PDBu binding assays (11) appeared to confirm the congruence of the major peak and the PDBu activity in both cases.

We then attempted to reisolate compound **1** from NCI contract collections of *M. africana* by monitoring both anti-HIV and PDBu activity, as well as by ^{1}H-nmr and hplc comparison with the repository sample of compound **1**. Although triterpene nmr signals were evident in spectra of initial fractions from the crude extract, it became clear that the bioactivity and triterpene nmr spectra diverged as fractionation progressed. For example, an early fraction from the partition of the CCl_4 solubles on Sephadex LH-20

[1]Part 23 in the series "HIV-Inhibitory Natural Products." For part 22, see Erickson *et al.* (1).
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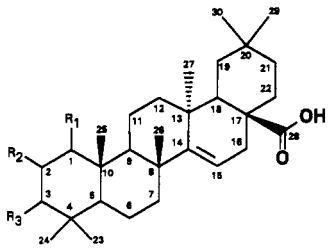

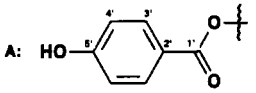

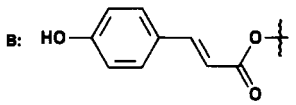

1 R_1=β-OH, R_2=H, R_3=β-A
2 R_1=R_2=H, R_3=β-A
3 R_1=H, R_2=α-OH, R_3=β-A
4 R_1=H, R_2=α-A, R_3 =β-OH
5 R_1=H, R_2=α-A, R_3=β-A
6 R_1=R_2=H, R_3=β-B
7 R_1=H, R_2=α-A, R_3=α-OH
8 R_1=β-OH, R_2=H, R_3=β-OH
9 R_1=β-OH, R_2=α-OH, R_3=H

displayed potent anti-HIV and PDBu activity, yet had none of the characteristic nmr signals of triterpene benzoate esters. This observation deepened our suspicion that non-triterpenoid compounds were responsible for the bioactivity. At this point, we became aware of a Japanese patent filing (12), which disclosed daphnane orthoesters with antitumor activity from the related species *M. membranacea*.

Careful chromatography of the repository sample of compound **1** on silica with a gradient of *i*-PrOH/MeOH in hexane separated several small peaks from the predominant triterpene. Online PDBu binding (11) clearly indicated that the minor peaks were responsible for the PDBu activity, and, by extension, the anti-HIV activity of this sample (13). The amount of these minor peaks was insufficient for further chemical characterization, but the major triterpene component was inactive in both assays.

To confirm these observations, we used the same gradient system to separate larger amounts of bioactive material from compound **1** obtained from our *M. africana* extract. KOH hydrolysis of this material under standard conditions and peracetylation with Ac_2O/pyridine gave a peracetate fraction that was examined by gc-eims. Although both this fraction and a sample of mezerein peracetate prepared under the same conditions gave complex gc chromatograms, the major peak in both samples showed very similar fragment ions. Coupled with the aforementioned disclosure of daphnanes in *M. membranacea* (12), we concluded that the bioactivity (PDBu, anti-HIV, and P-388) of compound **1** was most likely due to small amounts of very potent daphnane esters that were not originally separated from the major triterpene.

In the course of this work, both *M. africana* and *M. membranacea* extracts were thoroughly fractionated (14) in the search for the desired **1**, and six triterpene esters [**2**–**7**] were isolated in sufficient amounts and purity to permit nmr assignments and structure determinations. From *M. africana* we isolated **1**–**4**, while **4**–**7** were isolated from *M. membranacea*. Of these, **3**, **4**, **6**, and **7** are novel natural products.

Compounds **1** and **5** were identified by direct comparison to authentic samples. It should be noted that the structure of **1** has just been revised (9); the hydroxyl group previously reported to be at C-7 (β) has now been placed at C-1 (β). The other triterpene esters were clearly related, judging by visual inspection of their ^{1}H-nmr spectra. Hence, the ortho-coupled protons of the hydroxybenzoate moiety were common to all spectra, with **5** possessing two pairs of signals with similar 9 Hz couplings. Compound **6** exhibited, in addition, signals for a trans-olefinic system in the same region, indicating the presence of a cinnamate, rather than benzoate, ester.

The oxymethine signals and their coupling patterns were also informative as to the substitution positions. The acylation sites were determined from downfield shifts in

methine proton and carbon resonances induced by acylation (e.g., H-2/H-3 and C-2/C-3 in **3** and **4**) and confirmed by HMQC and HMBC nmr experiments. The H-3 protons in **3–5** and **7** appeared as simple doublets, in contrast to the doublets of doublets seen with no oxygen substituent at C-2, as in **1**, **2**, and **6**. A large 10–11 Hz trans-diaxial coupling in **3–5** justified assignment of 2α,3β-oxygenation in these compounds, while the small coupling between H-2 and H-3 in **7** suggested 2α,3α-substitution. Because the coupling constant for H-3 was 2.7 Hz, this had to represent an eq-ax coupling to H-2, which was a doublet of doublet of doublets, with 2.7, 4.3, and 12 Hz couplings. The difference nOe enhancement seen from H-2 to Me-23 required a trans-diaxial disposition. Thus, these data supported a 2α,3α-substitution. The ^{13}C-nmr spectra were all similar, with the exception of that of compound **7**, in which C-5 was shifted from 55–56 ppm to 48 ppm and C-25 was shifted from 15–17 ppm to 22 ppm, prompting closer investigation. The shift in the C-5 resonance could best be explained as a γ-gauche shielding effect due to the axial hydroxyl group at C-3. Numerous examples of oleanane triterpenes (differing from our taraxaranes in the C/D ring junction) have demonstrated consistent shifts of C-5 to higher field by either 1α- or 3α-hydroxyl groups (15–20). ^{13}C-Nmr assignments for **2–7** are presented in Table 1, and were supported by COSY, HMQC, and HMBC experiments.

The recent report, by groups at the University of Illinois at Chicago (UIC) and Research Triangle Institute (RTI), of HIV-1 reverse transcriptase inhibitory activity for several *Maprounea* triterpenes (21) prompted us to test compounds **1–7** in our reverse transcriptase inhibition assay. After detecting either no significant inhibition, or at most only marginal activity with a few of our triterpenes [**5–7**] (Table 2), we exchanged compounds with the UIC group for comparative testing of compounds **1–9** in both of the respective laboratories. The results (Table 2) suggested that inhibition of reverse transcriptase by these compounds is dependent on the particular assay conditions employed.

This investigation emphasizes the need for caution in interpreting bioassay-guided fractionation results for members of the family Euphorbiaceae, which can contain phorbol esters potently active in many different bioassays. Although phorbol esters had not been reported previously from the genus *Maprounea*, with the exception of Ref. (12), the most current taxonomic assignment of the genus places it in the subfamily Euphorbioideae and tribe Hippomaneae, in which there are many genera (e.g., *Excoecaria, Homalanthus, Sapium,* and *Stillingia*) that have been reported to contain phorbol esters (13, 22–25). Our previous survey of PDBu activity in the Euphorbiaceae showed that PDBu activity, and presumably phorbol esters, are widespread in only two of the five subfamilies of the Euphorbiaceae (2).

We have demonstrated in this study that the triterpene esters initially thought to be responsible for anti-HIV and phorbol ester receptor-binding activities of these *Maprounea* extracts were actually inactive in both respects, and that small quantities of daphnane diterpenes are the likely source of this bioactivity.

Triterpenes **1–9** gave markedly different results in RT inhibition assays performed in two different laboratories, even though the same enzyme source and generally similar assay conditions were used. These data suggest that relatively subtle variations [e.g., template primer, see Refs. (26–30)] in assay conditions may have led to contrasting results within this series.

The novel triterpenes present some interesting variations on familiar structural types. The 2,3-dihydroxy substitution pattern of compounds **3**, **4**, **5**, and **7** has precedent in sebiferenic acid (2 α-hydroxyaleuritolic acid), obtained from *Sapium sebiferum* (25). The 3α-OH substitution is not unknown (15–20), but is rare.

TABLE 1. ^{13}C-Nmr Data for Compounds **2–7**.[a]

Carbon	Compound					
	2	**3**	**4**	**5**	**6**	**7**
1	37.8	46.8	43.7	43.7	37.8	37.8
2	23.9	66.9	73.6	71.1	23.9	71.5
3	81.8	84.6	80.8	81.2	81.5	77.0
4	39.3[b]	40.0[b]	40.2	40.1[b]	39.3[b]	39.1
5	56.1	55.6	55.2	55.8	56.1	48.8[b]
6	19.0	18.9	19.1	19.0	19.0	18.7
7	41.3	41.0	41.3	41.2	41.3	41.3
8	38.4[b]	39.2[b]	39.5	39.5[b]	38.3[b]	39.7
9	49.5	50.5	49.4	49.8	49.4	48.6[b]
10	38.3[b]	39.1	39.2	39.3[b]	38.2[b]	39.5
11	17.7	17.6	17.8	17.8	17.7	17.7
12	35.8	33.5	33.6	33.6	35.8	33.7
13	37.7	37.6	37.7	37.7	37.7	37.7
14	162.0[c]	161.3	161.4	160.4[c]	160.7	160.7
15	117.1	117.1	116.5	117.3	117.1	117.1
16	32.1	31.9	32.1	32.1	32.1	32.1
17	51.3	49.0	50.5	51.3	51.3	51.3
18	42.1	41.3	42.1	42.0	42.0	42.0
19	34.1	35.7	35.8	35.8	34.1	35.8
20	29.5	29.4	29.5	29.5	29.5	29.5
21	33.8	33.9	34.1	34.1	33.7	34.1
22	31.4	31.2	31.4	31.4	31.4	31.4
23	28.3	28.6	28.6[b]	28.6[d]	28.2	28.6
24	17.1	17.9	16.8[c]	17.9	16.9	22.0
25	15.7	16.7	16.7[c]	16.7	15.7	16.6
26	26.1	26.1	26.0	26.1	26.1	26.2
27	22.6	22.5	22.6	22.6	22.5	22.4
28	181.2	181.0	180.4	181.2	181.2	181.2
29	32.3	32.1	32.3	32.3	32.3	32.3
30	29.0	28.8	28.9[b]	28.9[d]	29.0	28.9
1′	167.4	168.1	167.0	167.1, 167.4	168.4	167.1
2′	122.2	121.8	121.2	121.4, 121.5	115.3	121.9
3′	132.0	132.0	131.4	132.1	145.2	132.2
4′	115.5	115.3	114.7	115.3, 115.4	126.4	115.4
5′	160.7[c]	160.6	159.8	162.0[c] 162.0[c]	130.3	162.1
6′					116.1	
7′					159.8	

[a]In $CDCl_3$-d_4-MeOH (2:1) at 125 MHz.
[b–d]Assignments for values in the same column bearing the same superscript may be reversed.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All nmr spectra were obtained in a 2:1 mixture of $CDCl_3$-CD_3OD, referenced to the MeOH solvent multiplet at δ 3.30 and the carbon solvent resonance at δ 49.0, at 500 MHz for proton nmr and 125 MHz for carbon nmr on a Varian VXR-500 spectrometer. Mezerein was obtained from LC Services, Inc. (Boston, MA). Hplc was performed using a Waters 600 solvent delivery module equipped with a Waters 990 diode-array detector. Larger-scale hplc was accomplished using a Waters Delta-Prep unit with a variable wavelength uv detector.

PLANT MATERIAL.—*Maprounea africana* Muell.-Arg. samples were collected in Park Manovo-Gounda-

TABLE 2. HIV-1 Reverse Transcriptase Inhibitory Activity of *Maprounea* Triterpenes.

Compound	Source	IC_{50} NCI Assay	IC_{50} UIC Assay
1	UIC	>100 μM	3.7 μM[a]
2	NCI	200 μM	10.6 μM
3	NCI	>200 μM	14.4 μM
4	NCI	>200 μM	52 μM
5a	NCI	60 μM	7.9 μM
5b	UIC	>100 μM	3.7 μM[a]
6	NCI	60 μM	11.5 μM
7	NCI	40 μM	15 μM
8	UIC	stimulates @ 100 μM	4.9 μM[a]
9	UIC	>100 μM	3.4 μM[a]

[a]See Pengsuparp *et al.* (21).

St. Floris, Central African Republic, by John Michael Fay (Fay 8002) on 25 May 1987, at an elevation of 600 meters, in transitional woodland, sandy soil, close to watercourses. Leaf, bark, wood, and root samples were collected and extracted separately. *M. membrancea* Pax & K. Hoffman was collected and identified by Duncan W. Thomas (J. Nemba, P. Mambo, D.W. Thomas, and C. Strohmeier 794) on 18 February 1988, in Littoral Province, Cameroon, 5 km south of Dibombari, near Bonambwasse village, at sea level in secondary forest and overgrown *Elaeis* plantation. Leaf, bark, wood, and root samples were collected and extracted separately. Vouchers for both collections are deposited in the Smithsonian Institution under the numbers indicated in parentheses.

EXTRACTION AND ISOLATION.—The separate plant parts were air-dried in the field, ground, and extracted with CH_2Cl_2-MeOH (1:1, v/v), followed by a MeOH rinse, in percolators as previously described (32). The yield of the organic extracts for these parts was 25.15 g from 457 g dry *M. africana* root (5.5%) and 22.47 g from 589 g dry *M. membranacea* bark (3.8%). The roots of *M. africana* and the bark of *M. membranacea* possessed the most potent PDBu activity (IC_{50} values of 1.2 and 2.5 μg/ml, respectively) observed in the various plant parts and were, therefore, selected for further study.

Analytical-scale hplc was done on a silica column (Rainin Dynamax, 4.6×250 mm i.d., 8 μm, 60 Å) using a gradient of hexane/*i*-PrOH/MeOH at a flow rate of 1 ml/min. Samples of 5 μg were injected. Initial conditions were 90% hexane-10% *i*-PrOH for 2 min, then a linear gradient to 60% hexane, 35% *i*-PrOH, 5% MeOH at 8 min, then 2 min isocratic at final conditions. Fifteen-drop fractions were collected in glass test tubes for the PDBu-binding assay and each fraction was evaporated *in vacuo* before running the assay *in situ* (11).

Triterpenes from M. africana.—The crude organic extract (15.7 g) was partitioned between 500 ml 90% aqueous MeOH and 500 ml hexane (3×); then the aqueous MeOH phase was adjusted to 80% MeOH and extracted with 500 ml CCl_4 (3×). The aqueous MeOH was finally adjusted to 60% MeOH and extracted with $CHCl_3$ (3×). The weights of the fractions were hexane 916 mg; CCl_4 758 mg; $CHCl_3$ 1.131 g; and MeOH/H_2O 12.0 g. Anti-HIV (EC_{50} 4.3 μg/ml) and PDBu (98% at 0.5 μg/ml) activities were concentrated in the CCl_4 fraction. The CCl_4 fraction was permeated through Sephadex LH-20 in CH_2Cl_2-MeOH (1:1) to yield eight fractions. The middle two fractions were the largest and also had the most potent activity in both assays. The larger of these two fractions (463 mg) showed the higher anti-HIV activity (EC_{50} 0.078 μg/ml), and clearly had a triterpenoid nmr spectrum. This fraction was further separated by hplc on silica (4.1×25 cm, 8 μm, Rainin Dynamax) using hexane-*i*-PrOH (19:1). The first eluting fractions (11 and 17 mg) afforded bershacolone, an inactive, novel cyclobutene-containing diterpene (31), as well as 5.4 mg of compound **2**. Koumbalones A and B (32) were isolated from later fractions in the same sequence. A further fraction from silica chromatography (55 mg) was shown to contain small amounts of compound **1** by nmr analysis, as well as several other triterpenes. Silica hplc (1×25 cm i.d., 8 μm, Rainin Dynamax) using hexane-*i*-PrOH (97:3) yielded a 14-mg fraction that was further chromatographed on a Biotage Unisphere column using CH_3CN-H_2O (11:9), a separation that was previously reported from this laboratory (14). This separation yielded 5.2 mg of compound **3**, 2.8 mg of compound **1**, and 3.3 mg of compound **4**.

Triterpenes from M. membranacea.—The crude organic extract (5.1 g) was partitioned by the same

scheme described above using 500 ml (×2) each to give hexane (418 mg), CCl_4 (320 mg), $CHCl_3$ (304 mg), and aqueous MeOH (3.3 g) fractions. The bulk of the anti-HIV activity (EC_{50} 8.5 μg/ml) and PDBu displacement (53% at 50 μg/ml) was found in the CCl_4 fraction. This CCl_4 fraction was flash-chromatographed on Si gel using a gradient from hexane through EtOAc-MeOH (1:1) to MeOH. Fractions were pooled according to silica tlc (hexane-EtOAc, 1:1). Anti-HIV and PDBu activity occurred in late eluting, polar fractions. An earlier eluting, inactive fraction of 22 mg consisted of triterpenes by ^{1}H-nmr spectroscopy and was purified using normal-phase silica hplc (Rainin Dynamax, 4.1×25 cm i.d.) with 50 ml/min hexane-*i*-PrOH (97:3) to yield 7.2 mg of compound **6**. Yet another inactive flash chromatography fraction of intermediate polarity (138 mg) was chromatographed on the same silica column using hexane-EtOAc (7:3) to give 6.2 mg of compound **7**, 25.6 mg of compound **4**, and 36.8 mg of compound **5**. The most polar fractions yielded further small amounts of compound **5**. Fractions having anti-HIV and PDBu activity were not further characterized due to their complex nmr spectra and extremely small masses.

Compound **1** [*1β-hydroxyaleuritolic acid 3*-p-*hydroxybenzoate (NSC#308607)*].—$C_{37}H_{52}O_6$; uv (EtOH) λ max 259 nm (log ε 4.19); eims *m/z* 592 (0.5), 548 (9), 363 (9), 234 (39), 189 (52), 121 (100); hreims *m/z* 592.3765, calcd for $C_{37}H_{52}O_6$ 592.3764, Δ=0.1; ^{1}H nmr δ 7.83 (2H, d, *J*=8.7 Hz, H-3′), 6.80 (2H, d, *J*=8.7 Hz, H-4′), 5.53 (1H, dd, *J*=3.5 and 7.9 Hz, H-15), 4.59 (1H, dd, *J*=4.2 and 11.9 Hz, H-3), 3.46 (1H, dd, *J*=4.3 and 11.2 Hz, H-1), 2.34 (1H, dd, *J*=7.4 and 13.5 Hz, H-16), 2.27 (1H, dd, *J*=3.2 and 14.0 Hz, H-18), 0.99 (3H, s, H-25), 0.96 (3H, s, H-24), 0.95 (3H, s, H-26), 0.94 (3H, s, H-27), 0.90 (3H, s, H-29), 0.88 (3H, s, H-30), 0.85 (3H, s, H-23); ^{13}C nmr ($CDCl_3$-d_4-MeOH, 2:1, 125 MHz) δ 181.2 (C, C-28), 168.1 (C, C-1′), 162.2 (C, C-5′), 160.7 (C, C-14), 132.1 (2C, each CH, C-3′, C-5′), 121.9 (C, C-2′), 117.1 (CH, C-15), 115.5 (2C, each CH, C-4′, C-6′), 78.3 (CH, C-3), 78.0 (CH, C-1), 53.6 (CH, C-5), 51.9 (CH, C-9), 51.2 (C, C-17), 44.0, 42.1 (CH, C-18), 41.2, 39.8, 38.3 (C, C-4), 37.4, 35.9, 34.2, 34.1, 34.0 (CH_2, C-12), 32.3 (Me, C-29), 32.1 (CH_2, C-16), 31.4 (CH_2, C-22), 29.5 (CH_2, C-19), 28.9 (Me, C-30), 28.2 (Me, C-23), 26.0 (Me, C-26), 22.5 (Me, C-27), 20.8 (CH_2, C-21), 18.5 (CH_2), 16.7 (Me, C-24), 11.7 (Me, C-25).

Compound **2** [*aleuritolic acid 3*-p-*hydroxybenzoate*].—$C_{37}H_{52}O_5$; uv (EtOH) λ max 258 nm (4.19); eims *m/z* 576 (3), 530 (15), 438 (15), 234 (56), 189 (56), 121 (100); hreims *m/z* 576.3792, calcd for $C_{37}H_{52}O_5$ 576.3815, Δ=2.3; ^{1}H nmr δ 7.84 (2H, d, *J*=8.6 Hz, H-3′), 6.79 (2H, d, *J*=8.7 Hz, H-4′), 5.52 (1H, dd, *J*=3.4 and 8.1 Hz, H-15), 4.59 (1H, dd, H-3), 2.34 (1H, ddd, *J*=1.3, 7.9, and 14.5 Hz, H-16), 2.28 (1H, ddd, *J*=0.8, 3.5, and 13.9 Hz, H-18), 0.97 (3H, s), 0.94 (6H, s), 0.91 (3H, s), 0.90 (3H, s), 0.88 (3H, s), 0.86 (3H, s); ^{13}C-nmr data, see Table 1.

Compound **3** [*2α-hydroxyaleuritolic acid 3*-p-*hydroxybenzoate*].—$C_{37}H_{52}O_6$; uv (EtOH) λ max 259 nm (3.97); eims *m/z* 592 (3), 546 (6), 393 (7), 359 (40), 203 (35), 189 (43), 121 (100); hreims *m/z* 592.3766, calcd for $C_{37}H_{52}O_6$ 592.3764, Δ=0.2; ^{1}H nmr δ 7.95 (1H, d, *J*=9 Hz, H-3′), 6.86 (1H, d, *J*=9 Hz, H-4′), 5.58 (1H, dd, *J*=8.0 and 3.2 Hz, H-15), 4.69 (1H, d, *J*=10.5 Hz, H-3), 3.91 (1H, dt, *J*=10.5 and 4.8 Hz, H-2), 2.40 (1H, ddd, *J*=1, 3.2, and 14 Hz, H-16a), 2.35 (1H, dd, *J*=2 and 13 Hz, H-18), 2.06 (1H, dd, *J*=12.5 and 4.8 Hz, H-1a), 2.01 (1H, dt, *J*=13 and 2.5 Hz, H-7), 1.95 (1H, dd, *J*=3 and 14 Hz, H-16b), 1.06 (3H, s, H-25), 1.02 (3H, s, H-24), 1.00 (3H, s, H-26), 0.97 (3H, s, H-27), 0.96 (3H, s, H-29), 0.94 (3H, s, H-30), 0.91 (3H, s, H-23); ^{13}C-nmr data, see Table 1.

Compound **4** [*2α-hydroxyaleuritolic acid 2*-p-*hydroxybenzoate*].—$C_{37}H_{52}O_6$; uv (EtOH) λ max 259 nm (4.25); fabms *m/z* 593 (17), 437 (10), 409 (52), 249 (16), 205 (25), 189 (33), 155 (63), 139 (71), 121 (100); hrfabms *m/z* 593.3716 $(MH)^+$ calcd for $C_{37}H_{53}O_6$ 593.3841; ms/ms *m/z* 593→409; eims *m/z* 592 (0.6), 546 (3), 410 (9), 359 (19), 234 (20), 189 (61), 121 (100); hreims *m/z* 592.3708, calcd for $C_{37}H_{52}O_6$ 592.3764, Δ=5.5; ^{1}H nmr δ 7.87 (2H, d, *J*=8.5 Hz, H-3′), 6.78 (2H, dd, *J*=8.5 and 1.5 Hz, H-4′), 5.52 (1H, dd, *J*=3.7 and 7.8 Hz, H-15), 5.11 (1H, dt, *J*=4.5 and 10.5 Hz, H-2), 3.26 (1H, d, *J*=10.5 Hz, H-3), 2.34 (1H, dd, *J*=7.8 and 14 Hz, H-16), 2.28 (1H, br d, *J*=13.5 Hz, H-18), 1.05 (3H, s, H-24), 1.01 (3H, s, H-23), 0.94 (3H, s, H-26), 0.89 (6H, s, H-27, H-29), 0.87 (6H, s, H-25, H-30); ^{13}C-nmr data, see Table 1.

Compound **5** [*2α-hydroxyaleuritolic acid 2,3*-bis(p-*hydroxybenzoate*)].—$C_{44}H_{56}O_8$; uv (EtOH) λ max 259 nm (4.23); eims *m/z* 712 (0.1), 668 (0.4), 547 (1), 408 (6), 248 (22), 203 (36), 189 (50), 121 (100); hreims *m/z* 712.3963, calcd for $C_{44}H_{56}O_8$ 712.3975, Δ=1.2; ^{1}H nmr δ 7.74 (1H, d, *J*=8.5 Hz, H-3′), 7.69 (1H, d, *J*=8.5 Hz, H-3″), 6.71 (1H, d, *J*=8.5 Hz, H-4′), 6.66 (1H, d, *J*=8.5 Hz, H-4″), 5.59 (1H, dd, *J*=3.5 and 8.6 Hz, H-15), 5.39 (1H, dt, *J*=4.4 and 10.6 Hz, H-2), 5.11 (1H, d, *J*=10.6 Hz, H-3), 2.29 (1H, dd, *J*=3.5 and 13.5 Hz, H-18), 1.91 (1H, dd, *J*=13.5 and 14 Hz, H-16), 1.14 (3H, s, H-25), 1.04 (3H, s, H-24), 0.97 (3H, s, H-26), 0.93 (3H, s, H-23), 0.90 (3H, s, H-27), 0.895 (3H, s, H-29), 0.88 (3H, s, H-30); ^{13}C-nmr data, see Table 1.

Compound **6** [*aleuritolic acid 3*-p-*hydroxycinnamate*].—$C_{39}H_{54}O_5$; uv (EtOH) λ max 311 nm (4.22); eims *m/z* 602 (0.5), 558 (4), 438 (11), 392 (22), 248 (42), 189 (100), 120 (53); hreims *m/z* 602.3955, calcd for $C_{39}H_{54}O_5$ 602.3971, Δ=1.6; fabms (negative-ion mode, glycerol matrix) *m/z* 601 (M-H), 513, 459, 367;

^{1}H nmr δ 7.54 (1H, d, J=15.9 Hz, H-3′), 7.37 (2H, d, J=8.8 Hz, H-5′), 6.78 (2H, d, J=8.7 Hz, H-6′), 6.22 (1H, d, J=15.8 Hz, H-2′), 5.52 (1H, dd, J=3.4 and 8.0 Hz, H-15), 4.52 (1H, dd, H-3), 2.34 (1H, ddd, J=1.4, 8.0, and 14.4 Hz, H-16), 2.28 (1H, ddd, J=1.1, 3.2, and 13.8 Hz, H-18), 0.94 (3H, s), 0.93 (3H, s), 0.91 (6H, s), 0.90 (3H, s), 0.88 (3H, s), 0.85 (3H, s); ^{13}C-nmr data, see Table 1.

Compound **7** [*3α-hydroxyaleuritolic acid 2β-p-hydroxybenzoate*].—$C_{37}H_{52}O_6$; uv (EtOH) λ max 259 nm (4.12); eims *m/z* 592 (1), 590 (3), 546 (4), 452 (9), 359 (7), 205 (26), 189 (42), 121 (100); hreims *m/z* 592.3728, calcd for $C_{37}H_{52}O_6$ 592.3764, Δ=3.6; ^{1}H nmr δ 7.88 (2H, d, J=8.5 Hz, H-3′), 6.78 (2H, d, J=8.5 Hz, H-4′), 5.51 (1H, dd, J=3.5 and 8 Hz, H-15), 5.34 (1H, ddd, J=2.7, 4.3, and 12 Hz, H-2), 3.53 (1H, br d, J=2.7 Hz, H-3), 2.33 (1H, ddd, J=1, 8.5, and 14 Hz, H-16β), 2.27 (1H, dd, J=3 and 13 Hz, H-18), 1.89 (1H, dd, J=3.5 and 14 Hz, H-16α), 1.03 (3H, s, H-23), 0.96 (3H, s, H-24), 0.94 (3H, s, H-26), 0.93 (3H, s, H-25), 0.894 (3H, s, H-27), 0.889 (3H, s, H-29), 0.87 (3H, s, H-30); ^{13}C-nmr data, see Table 1.

HYDROLYSIS, ACETYLATION, AND GC-MS.—The samples for gc-ms analysis (1 mg) were hydrolyzed for 30 min in 0.5 N KOH, diluted with H_2O, and extracted with CH_2Cl_2 (3×), then evaporated and dried *in vacuo*. Pyridine and Ac_2O (1 ml each) were added and the reaction was allowed to proceed for 90 min, at which time the reaction was quenched with 2 ml H_2O, extracted with CH_2Cl_2 (3×), and evaporated to a pyridine solution, which was injected directly on to the gas chromatograph.

Gc-ms was done using a DB-1 capillary column (0.32 mm×30 m), 25 μm film coating, He carrier gas, on-column injection with a temperature program from 105° to 200° at 20°/min, then to 300° at 8°/min. Eims were obtained at 70 eV on a VG 70–250 instrument.

BIOASSAYS.—Anti-HIV primary screens were performed as described previously (10). Phorbol ester receptor-binding assays (displacement of [^{3}H]-PDBU) were performed as described (2). Triterpenes **1–9** were assayed for inhibitory activity against recombinant HIV-1 reverse transcriptase as described (33–35). Pure compounds were diluted in virus disruption buffer (VDB) containing 50 mM *tris* pH 7.9, 0.15 mg/ml dithiothreitol (DDT), and 0.1% Triton X-100. Serial dilutions of **1–9** were made in individual wells of a 96-well V-bottom plate. Recombinant enzyme was diluted in VDB and added to appropriate wells (50 μl/well). RT control wells received only VDB. Triplicate 10-μl aliquots from each well were each added to 30 μl cocktail containing 2 μl of 1 M *tris* pH 7.9, 1 μl of 3 M KCl, 5 μl of 3 mg/ml DTT, 5 μl of 0.1 M magnesium acetate, 10 μl of poly r(A)-p(dT)$_{12-28}$ (2 units/ml Pharmacia Biochemicals, Piscataway, NJ), 6.5 μl distilled H_2O, 0.5 μl distilled H_2O, 0.5 μl of 10% Triton X-100 and 10 μl of [^{3}H]-TTP (tritiated thymidine triphosphate) (16.56 Ci/mmol). The [^{3}H]-TTP (New England Nuclear-DuPont) was acquired as a 5mCi/ml solution of approximately 80 Ci/mmol preparation; it was evaporated to dryness under N_2 and resuspended in 1×RT buffer. Samples were incubated for 30 min at 37°, harvested onto DE-81 ion-exchange paper (Schleicher and Scheuel Keene, NH) and allowed to adsorb. Following extensive washing with 5% Na_2PO_4 (dibasic), the membranes were dried and counted in a liquid scintillation counter (Beckman LS6000LL, Beckman Instruments).

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