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ISOLATION AND STRUCTURAL ELUCIDATION OF PENTACYCLIC TRITERPENOIDS FROM *MAPROUNEA AFRICANA*

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ABSTRACT.—Pentacyclic triterpenoids based on the taraxer-14-ene skeleton with a C-28 attached carboxylic acid group have been isolated from the roots of *Maprounea africana*. These compounds were identified as 1β,2α-dihydroxyaleuritolic acid 2,3-*bis*-*p*-hydroxybenzoate [**1**], 2α-hydroxyaleuritolic acid 3-*p*-hydroxybenzoate [**2**], 2α-hydroxyaleuritolic acid 2,3-*bis*-*p*-hydroxybenzoate [**4**], aleuritolic acid 3-*p*-hydroxybenzoate [**5**], aleuritolic acid [**6**], and aleuritolic acid 3-acetate [**7**]. Compounds **1** and **2** are new triterpene esters. Compound **3** was previously reported as 7β-hydroxymaprounic acid 3-*p*-hydroxybenzoate [**13**]. However, based on detailed nmr studies, its structure has been revised.

Maprounea africana Muell. Arg. (Euphorbiaceae) is a woody species native to central and eastern Africa that has a reputation of being a toxic plant (1). Previous phytochemical investigations on this plant have involved the isolation or analysis of cucurbitacins (2), hydroxylated taraxerane-type triterpenes (3–5), and an unusual cyclobutene ring-containing macrocyclic diterpenoid (6).

As part of a search for naturally occurring cancer chemopreventive agents, we have investigated a 90% MeOH extract of the roots of *M. africana*. This extract and a constituent triterpenoid have been reported previously to affect phorbol ester-mediated events related to tumor promotion (7). However, these biologic responses may not actually be due to the triterpenoid components of the plant, but rather, chemical contaminants that co-purify with the triterpenes may mediate the observed activities, possibly daphnane esters (8). On the other hand, triterpenoid isolates from *M. africana* have been shown clearly to inhibit the catalytic activity of HIV-1 reverse transcriptase (9), although this inhibitory activity may not be of therapeutic value, since kinetic analysis has demonstrated recently that the response is not specific (10).

Some of the compounds described herein that were isolated and characterized from the 90% methanol extract of *M. africana* roots have been previously reported as derivatives of maprounic acid (3,4). However, the structure of maprounic acid was later revised on the basis of single-crystal X-ray crystallography and found to be identical to aleuritolic acid (4). We report herein the structural determination of the new triterpenoids **1** and **2**, the revision of structure of compound **13** to **3**, and the full characterization of the previously reported compounds **4–7**, along with several derivatives [**8–12**].

RESULTS AND DISCUSSION

The residue from fractions 4–8 obtained by Craig countercurrent distribution of the

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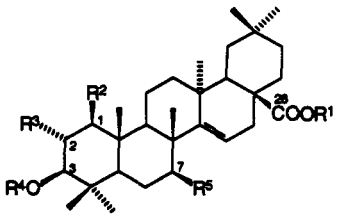

Compound	R^1	R^2	R^3	R^4	R^5
1	H	OH	4″-OHC_6H_4COO	4′-OHC_6H_4CO	H
2	H	H	OH	4′-OHC_6H_4CO	H
3	H	OH	H	4′-OHC_6H_4CO	H
4	H	H	4″-OHC_6H_4COO	4′-OHC_6H_4CO	H
5	H	H	H	4′-OHC_6H_4CO	H
6	H	H	H	H	H
7	H	H	H	Ac	H
8	H	OH	H	H	H
9	Me	OH	H	4′-$MeOC_6H_4CO$	H
10	H	OAc	H	4′-$AcOC_6H_4CO$	H
11	H	H	OH	H	H
12	Me	H	4″-$MeOC_6H_4COO$	4′-$MeOC_6H_4CO$	H
13	H	H	H	4′-OHC_6H_4CO	OH

90% MeOH extract of *M. africana* was chromatographed repeatedly over Si gel followed by prep. hplc in some cases (see Experimental), leading to the isolation of seven pentacyclic triterpenes, **1–7**. Compounds **1** and **2** are new constituents. The structures of these compounds were elucidated by means of spectroscopic methods (in particular 2D nmr) and derivatization. The complete ^{13}C-nmr assignments for the triterpenes **1–7** and their derivatives **8–12** are given in Table 1. These ^{13}C-nmr assignments were made using a combination of ^{1}H-^{13}C HETCOR, DEPT, selective INEPT (11), and HMBC experiments and also by comparison with ^{13}C-nmr data of 3-acetylaleuritolic acid (=maprounic acid acetate) [**7**], reported previously by McLean *et al.* (12).

Compound **1**, isolated as an amorphous solid, exhibited a negative hrfabms molecular ion, $[M-H]^-$ at *m/z* 727.3846, corresponding to a molecular formula of $C_{44}H_{55}O_9$. The molecular formula of **1** differed from that of **4** (3,4) by the presence of an additional oxygen atom. Comparison of the spectral properties [uv, ir, ^{1}H- and ^{13}C-nmr (Table 1)] of **1** with those of 2α-hydroxyaleuritolic acid 2,3-*bis*-*p*-hydroxybenzoate [**4**], suggested that **1** is closely related to **4** except for the presence of a hydroxyl group in ring A of **1**. The ^{1}H-nmr spectrum of **1** displayed seven Me singlets at δ 1.40 (H_3-25), 1.24 (H_3-26), 1.22 (H_3-27), 1.16 (H_3-24), 1.07 (H_3-30), 1.00 (H_3-23), and 0.99 (H_3-29), one methine proton at δ 3.89 (H-1α), two ester methine protons at δ 6.13 (H-2β) and 5.52 (H-3α), and an olefinic proton at δ 5.88 (H-15). Aromatic protons at δ 8.23 and 8.14 (each 2H, H-2′, -6′, and H-2″, -6″), 7.03 and 6.95 (each 2H, H-3′, -5′, and H-3″, -5″), representing two AA′BB′ systems, were in conformity with the presence of two *p*-hydroxybenzoyl ester moieties. In its ^{1}H-nmr spectrum, the downfield shift (Δ 0.34 ppm) of H-2β in **1** relative to **4** suggested the hydroxyl group in **1** was located at C-1. In the ^{1}H-^{1}H COSY nmr spectrum of **1**, mutual couplings between H-2 (δ 6.13) and H-1 (δ 3.89) and H-2 and H-3 (δ 5.52), confirmed the location of a hydroxyl group at C-1. The coupling constants of H-1α and H-3α (each d, J=10 Hz) suggested their trans-diaxial relationship with H-2β (t, J=10 Hz). The assignment of the ^{1}H-nmr spectrum of **1** was supported by its ^{1}H-^{1}H COSY and ^{1}H-^{13}C HETCOR experiments.

The ^{13}C-nmr spectrum of **1** exhibited signals for seven CH_3, eight CH_2, fifteen CH, and fourteen non-protonated carbons, further confirming its molecular formula of

TABLE 1. ^{13}C-Nmr Spectral Data (δ) of Compounds **1–12**.

Carbon	Compound											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**[a]	**10**[a]	**11**	**12**[a]
C-1	80.6	47.7	77.6	43.7	37.4	38.2	37.4	78.4	77.7	79.3	47.0	43.4
C-2	74.7	66.4	35.3	70.8	24.0	28.1	23.8	39.5	35.5	29.9	68.5	70.7
C-3	79.1	85.1	78.3	80.8	80.9	78.1	80.7	75.6	78.0	77.2	83.7	80.6
C-4	39.5	39.9	38.4	39.9	38.1	39.4	37.8	39.5	38.7	38.0	39.7	39.7
C-5	52.8	55.5	53.5	55.3	55.7	56.0	55.6	53.8	53.2	52.9	56.0	55.3
C-6	18.5	19.0	18.6	18.8	18.8	19.1	18.8	18.8	18.2	18.1	17.9	17.5
C-7	41.0	41.1	41.3	41.0	41.2	41.5	41.2	41.5	40.8	41.3	41.3	40.9
C-8	39.9	39.2	39.9	39.3	39.2	39.3	39.2	39.9	38.5	39.3	39.3	39.0
C-9	50.7	49.4	50.7	49.2	49.3	49.6	49.3	50.8	50.1	49.6	49.5	49.1
C-10	44.8	39.2	44.2	39.4	38.3	38.3	38.0	44.2	43.7	40.4	39.3	39.2
C-11	21.1	17.9	21.3	17.9	17.8	17.8	17.7	21.3	20.7	19.0	19.1	18.7
C-12	34.4	34.4	34.5	34.4	34.4	34.3[c]	34.3	34.5	33.9	33.7	34.3	33.8
C-13	37.5	37.7	37.6	37.8	37.7	37.8	37.7	37.6	37.1	36.9	37.7	37.4
C-14	160.6	160.4	160.9	160.3	160.5	160.7	160.5	161.0	160.3	160.0	160.6	160.3
C-15	117.4	117.3	117.3	117.4	117.2	117.1	117.2	117.1	116.9	117.2	117.0	116.8
C-16	32.7	32.6	32.7	32.5	32.5	32.3	32.5	32.6	32.1	31.9	32.5	32.1
C-17	51.1	51.2	51.2	51.2	51.2	51.2	51.2	51.1	51.3	51.4	51.2	51.4
C-18	42.3	42.2	42.4	42.2	42.2	42.2	42.2	42.3	41.9	42.3	42.1	41.8
C-19	36.0	35.9	36.0	35.9	35.9	35.9	35.9	35.9	35.5	35.3	35.8	35.5
C-20	29.6	29.6	29.6	29.6	29.6	29.3	29.6	29.6	29.3	29.3	29.9	29.7
C-21	34.4	33.8	34.4	33.7	33.8	33.9	33.8	34.3	33.8	33.6	33.8	33.3
C-22	31.7	31.7	31.7	31.7	31.6	31.6	31.6	31.6	31.0	30.7	31.5	31.0
C-23	28.5	28.8	28.2	28.5	28.2	28.6	28.0	28.6	28.0	27.9	29.1	28.4
C-24	17.8	18.1	16.9	17.8	17.1	16.4	16.8	16.1	16.5	16.5	17.5	17.7
C-25	13.8	16.8	12.3	16.7	15.5	15.7	15.5	12.4	11.6	12.9	16.9	16.6
C-26	26.2	26.3	26.3	26.2	26.2	26.3	26.2	26.3	26.2	26.2	26.3	26.3
C-27	22.6	22.4	22.7	22.6	22.5	22.5	22.5	22.7	22.5	22.3	22.4	22.4
C-28	180.5	180.6	180.4	180.3	180.3	180.2	180.2	180.3	178.5	184.3	180.2	178.5
C-29[f]	32.3	32.4	32.4	32.3	32.3	32.5	32.3	32.3	31.7	31.4	32.3	31.7
C-30[f]	29.2	29.2	29.2	29.1	29.2	29.2	29.2	29.1	28.7	28.7	29.5	29.3

TABLE 1. Continued.

Carbon	Compound											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**[a]	**10**[a]	**11**	**12**[a]
C-1′	121.3[b]	122.5	122.2	121.6	122.3		C-3-OAc		122.9	128.0		122.6[b]
C-2′, -6′	132.4	132.5	132.4	132.4	132.4		170.6		131.6	131.1		131.6
C-3′, -5′	116.1[c]	116.1	116.3	116.2[b]	116.2		21.0		113.6	121.6		113.5
C-4′	163.1[d]	163.4	163.7	163.6	163.4				163.3	154.2		163.2
C-7′	166.6[e]	167.0	166.4	166.4[c]	166.4				165.9	164.9		166.0[c]
C-1″	122.0[b]			121.6						C-1-OAc		122.7[b]
C-2″, -6″	132.4			132.4						170.1		131.6
C-3″, -5″	115.8[c]			116.1[b]						21.7		113.5
C-4″	163.5[d]			163.6						C-4-OAc		163.2
C-7″	166.9[e]			166.7[c]						168.9		166.2[c]
C-28-OMe									51.7	21.1		51.7
C-4′-OMe									55.4			55.4[d]
C-4″-OMe												55.5[d]

[a]In $CDCl_3$, others in C_5D_5N.

[b-c]Assignments in the same column with the same superscript are interchangeable.

[f]The spectra provide no direct method for distinguishing C-29 from C-30 since they are present as a *gem*-dimethyl pair. Therefore, these assignments may be reversed.

$C_{44}H_{56}O_9$. Comparison of the ^{13}C-nmr resonances of **1** with those of **2** and **4** (Table 1) indicated that **1** bears a hydroxyl group at C-1. The downfield shifts (ca. Δ 36.9, 5.4, and 3.9 ppm, respectively) of the C-1, C-10, and C-2 resonances and the upfield shifts (ca. Δ 2.9, 2.5, and 1.7 ppm, respectively) of the C-25, C-5, and C-3 resonances in **1** relative to **4** further supported the fact that the hydroxyl group at C-1 was β-equatorially oriented (13). Thus, the structure of **1** was assigned as 1β,2α-dihydroxyaleuritolic acid 2,3-*bis*-*p*-hydroxybenzoate.

Compound **2**, an amorphous solid, showed a negative hrfabms molecular ion, $[M-H]^-$ at *m/z* 591.3685, corresponding to a molecular formula of $C_{37}H_{51}O_6$. Comparative ^{13}C-nmr spectral (Table 1) studies between **2** and **5**, revealed the presence of an additional hydroxyl group in ring A of **2**. The ^{1}H-nmr spectrum of **2** indicated the presence of a *p*-hydroxybenzoyl ester moiety [δ 8.30 and 7.18 (each 2H, H-2′, H-6′, and H-3′, H-5′)], seven Me singlets at δ 1.15 (H_3-26), 1.08 (H_3-30), 1.07 (H_3-27), 1.06 (H_3-24), 1.01 (H_3-25), 1.00 (H_3-29), and 0.98 (H_3-23), an olefinic proton at δ 5.81 (H-15), and two methine protons at δ 5.27 (H-3α) and 4.32 (H-2β). A ^{1}H-^{1}H COSY nmr experiment on **2** showed mutual couplings between H-2 and H_2-1 and H-2 and H-3, supporting the attachment of the hydroxyl group at C-2. In the ^{1}H-nmr spectrum of **2**, the coupling constants of H-2β (dt, J=4 and 10 Hz) and H-3α (d, J=10 Hz), suggested their trans-diaxial relationship, and that therefore the C-2 hydroxyl group is α-oriented. When the ^{13}C-nmr spectra were compared (Table 1), the downfield shifts (ca. Δ 42.4, 10.3, 4.2, 1.8, and 0.9 ppm, respectively) of the C-2, C-1, C-3, C-4, and C-10 resonances in **2** relative to **5**, were in conformity with the presence of a 2α-hydroxyl group. The structure of **2** was therefore established as 2α-hydroxyaleuritolic acid 3-*p*-hydroxybenzoate.

Compound **3**, isolated as an amorphous solid, exhibited a molecular ion at *m/z* 592.3766, as determined by hreims, corresponding a molecular formula of $C_{37}H_{52}O_6$, which is identical with that of 2α-hydroxyaleuritolic acid 3-*p*-hydroxybenzoate [**2**]. Analysis of the ^{1}H- and ^{13}C-nmr data (Table 1) and other spectroscopic parameters of **3**, suggested it was an isomer of **2**. The ^{1}H-nmr spectrum revealed seven Me singlets at δ 1.20 (H_3-26), 1.19 (H_3-25), 1.14 (H_3-27), 1.03 (H_3-24), 1.01 (3H, s, H_3-30), 0.94 (H_3-29), and 0.90 (H_3-23), two methine protons at δ 4.97 (H-3α) and 3.65 (H-1α), an olefinic proton at δ 5.81 (H-15), and aromatic protons at δ 8.21 and 7.17 (each 2H, H-2′, H-6′, and H-3′, H-5′), representing an AA′BB′ system.

Inspection of the ^{1}H-^{1}H COSY nmr data of **3** revealed mutual couplings of the 2α- (δ 2.41) and 2β- (δ 2.23) protons to the C-1 and C-3 methine protons. In the HOHAHA (14) nmr spectrum of **3**, the connectivities observed as a result of vicinal proton-proton coupling in ring A of **1**, at δ 3.65 (H-1α), 2.41 (H-2α), 2.23 (H-2β), and 4.97 (H-3α), were supportive of oxygenated functionalities being affixed to C-1 and C-3. The ^{1}H-nmr coupling constants of the H-1α (dd, J=11.5 and 4 Hz) and the H-3α (dd, J=12 and 4 Hz) methine protons indicated that they were both axial. The fact that a *p*-hydroxybenzoic ester substituent occurred in **3** at C-3 was established by analyzing its selective INEPT and ^{1}H-^{13}C FLOCK (15) nmr spectra. Thus, in a selective INEPT (11) nmr experiment of **3**, soft irradiation ($^3J_{CH}$=5 Hz) of the C-3 methine proton at δ 4.97 led to the enhancement of C-7′, C-4, C-23, and C-24 (δ 166.4, 38.4, 28.2, and 16.9), respectively. In the ^{1}H-^{13}C FLOCK nmr spectrum of **3**, the carbon signals at δ 78.3 (C-3) and 77.6 (C-1) revealed correlations with the 23- and 24-Me signals, and with the 25-Me signal, respectively. Comparative ^{13}C-nmr spectral (Table 1) studies on **3**, **5**, and **7** in conjunction with the C-1 to C-5, C-10, and C-25 resonances in 1β-hydroxy-3β-acetoxyolean-18-ene (16), also corroborated the presence of a 1β-hydroxyl group in **3**. This was supported by the downfield shifts [ca. Δ 40.2 (40.2), 11.3 (11.5), and 5.9 (6.2)

ppm, respectively] of the C-1, C-2, and C-10 resonances and upfield shifts [ca. Δ 3.2 (3.2), 2.6 (2.4), and 2.2 (2.1) ppm, respectively] of the C-25, C-3, and C-5 resonances in **3** relative to **5** and **7**, respectively. On hydrolysis with 6% aqueous KOH, compound **3** generated the product **8**, the 1β-OH derivative of "maprounic acid" [**6**] (3), a compound isolated from *M. africana* that was later shown by X-ray crystallography to have the same structure as the previously characterized compound, aleuritolic acid (4,17). Therefore, the structure of **3** was elucidated as 1β-hydroxyaleuritolic acid 3-*p*-hydroxybenzoate.

Compound **3** showed closely comparable physical and spectroscopic properties ([α]D, uv, and ir) with those reported previously for the compound identified as 7β-hydroxymaprounic acid 3-*p*-hydroxybenzoate [**13**] (3). In addition, the molecular ion at *m/z* 592 was the same. The latter compound **13** differed from **3** only in the position assigned to the β-hydroxyl group. The position of the 7β-hydroxyl group in **13** was previously reported primarily based on its mass spectral fragmentation patterns. Mass spectral fragmentation patterns have, in the past, been widely used for assigning molecular skeletons of triterpenes (15). However, this is not a completely reliable technique as demonstrated previously in the case of 3-acetylaleuritolic acid (=maprounic acid acetate) [**7**] (12). The ambiguity in the present case was solved by using 2D nmr (^{1}H-^{1}H COSY, ^{1}H-^{1}H HOHAHA, ^{1}H-^{13}C HETCOR, selective INEPT and HMBC) techniques extensively for compound **3** and it was proved that the β-hydroxyl group in **3** is affixed to C-1 instead of C-7, as in **13**. Hence the revised structure for the compound previously reported as **13** is 1β-hydroxyaleuritolic acid 3-*p*-hydroxybenzoate [**3**]. Compounds **4–6** have been previously isolated from *M. africana* in the R.T.I. laboratory and were reported as maprounic acid and its derivatives (3). However, compound **7** (12,19) is being reported for the first time from this plant. On hydrolysis with 6% aqueous KOH, compound **4** generated 2α-hydroxyaleuritolic acid [**11**], identical with sebiferenic acid (20).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured with Perkin-Elmer Model 141 and Model 241 polarimeters. Uv spectra were obtained on Beckman DU-7 and Varian 2290 uv-vis spectrometers, and ir spectra on a Midac Collegian Ft-ir spectrophotometer and a Perkin-Elmer 467 grating spectrometer. ^{1}H-Nmr, ^{13}C-nmr, DEPT, and ^{1}H-^{13}C FLOCK spectra were measured with TMS as the internal standard, employing a Varian XL-300 instrument operating at 300 MHz or 75.6 MHz and Bruker AMX 500 and 250 spectrometers operating at 500 MHz or 125 MHz and 250 MHz or 62.5 MHz. Selective INEPT nmr spectra were conducted on a Nicolet NT-360 spectrometer operating at 90.8 MHz. ^{1}H-^{1}H COSY, ^{1}H-^{13}C HETCOR, HMBC, and ^{1}H-^{1}H HOHAHA 2D nmr experiments were carried out on an Omega-GE 500 spectrometer and Bruker AMX 500 and Bruker AMX 250 spectrometers. Eims and fabms (low- and high-resolution) were performed on a Finnigan MAT-90 instrument and Associated Electrical Industries MS-902. Cc was performed using Si gel 60 (70–230 mesh, E. Merck, Darmstadt, Germany). Tlc was carried out on Merck aluminum-backed tlc sheets (Si gel F_{254}), with visualization using phosphomolybdate spraying reagent (5% phosphomolybidic acid in EtOH). Prep. tlc was performed on Merck Si gel plates (1 mm layer thickness). Prep. hplc was performed with a Waters Model prep-3000 equipped with a Lambda Model 481 LC spectrophotometer and a Waters 740 Data Module. A Dynamax C_{18} column (2.15×25 cm) and Si column (2.15×25 cm) were used for reversed-phase and normal phase, respectively.

PLANT MATERIAL.—As published previously by Wani *et al.* (3).

EXTRACTION AND FRACTIONATION.—The air-dried, milled roots of *M. africana* (2.4 kg) were extracted with 50% MeOH-$CHCl_3$ (2×2 liters) in a Soxhlet apparatus. The residue was further extracted successively with 80% MeOH-H_2O (2 liters) and 90% MeOH-H_2O (2 liters). The resultant extracts were combined, concentrated and partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ layer was dried, taken up in 90% MeOH/H_2O (1 liter) and defatted with *n*-hexane (2×1 liter). The aqueous MeOH was then concentrated under vacuum and lyophilized to a powder (37.7 g). The lyophilized power was then subjected to an 11-tube Craig

countercurrent distribution (ccd) procedure, using CCl_4-$CHCl_3$-MeOH-H_2O (35:15:40:10), as described previously (3,21).

The residue (7 g) from the ccd combined fractions 4–8, was chromatographed on a Si gel (800 g) column with $CHCl_3$ and increasing percentages of MeOH in $CHCl_3$ (0% to 30%). A total of 850 fractions of 20 ml each was collected and combined to produce four pooled fractions FI (2.5 g), FII (1.19 g), FIII (1.7 g) and FIV (0.74 g), based on their tlc profiles. Fraction FI (2.4 g) was repeatedly chromatographed over Si gel, with $CHCl_3$ and increasing amounts of MeOH (0.2 to 2%) to furnish fractions FI-1 (40 mg), FI-2 (60 mg), and FI-3 (240 mg). Compound **7** (20 mg, 0.001% w/w) was recrystallized with *n*-hexane/EtOAc from the solution of fraction FI-1. FI-2 (60 mg) was finally purified by normal-phase prep. hplc [isooctane-EtOH (9:1), 20 ml/min, uv 210 nm] to give pure compound **6** (30 mg, 0.001% w/w). FI-3 (240 mg) was further separated by cc (Si gel) eluting with $CHCl_3$ and increasing amounts of MeOH (0.1 to 0.5%), followed by reversed-phase prep. hplc [MeOH-H_2O (8.5:1.5), 15 ml/min, uv 254 nm] to afford pure compound **5** (15 mg, 0.001% w/w).

FIII (1.6 g) was subjected to further cc (Si gel, 160 g), eluting with $CHCl_3$ and increasing amounts of MeOH (0.2 to 3%) to yield fractions FIII-1 (194 mg), FIII-2 (150 mg), FIII-3 (160 mg), and FIII-4 (400 mg). Fractions FIII-1 (170 mg), FIII-2 (130 mg), and FIII-3 (120 mg) were finally purified by reversed-phase prep. hplc [MeOH-H_2O (8.5:1.5), 20 ml/min, uv 254 nm] to yield compounds **3** (100 mg, 0.004% w/w), **2** (60 mg, 0.003% w/w), and **4** (90 mg, 0.004% w/w), respectively, in pure form. FIII-3 (380 mg) was further separated over a C_{18} (40 g; Bakerband, 40 μm prep. lc packing) column, eluting with CH_3CN-H_2O (8.5:1.5) at a flow rate of 1 ml/min, followed by reversed-phase prep. hplc [MeOH-H_2O (8.5:1.5), 15 ml/min, uv 254 nm] to afford pure compound **1** (13 mg, 0.0005% w/w).

*1*β,*2*α-*Dihydroxyaleuritolic acid 2,3*-bis-p-*hydroxybenzoate* [**1**].—Amorphous solid; mp 280–284° (dec); $[\alpha]_D$ −23.1° (*c*=0.16, pyridine); uv λ max (MeOH) (log ε) 257 (4.51) nm; ir ν max (KBr) 3360, 1718, 1684, 1591, 1270, 1161 cm^{-1}; ^1H nmr (C_5D_5N, 500 MHz) δ 8.23 and 8.14 (each 2H, d, *J*=8.5 Hz, H-2′, -6′, and H-2″, -6″), 7.03 and 6.95 (each 2H, d, *J*=8.5 Hz, H-3′, -5′, and H-3″, -5″), 6.13 (1H, t, *J*=10 Hz, H-2β), 5.88 (1H, br d, *J*=5 Hz, H-15), 5.52 (1H, d, *J*=10 Hz, H-3α), 3.89 (1H, d, *J*=10 Hz, H-1α), 2.85 (2H, m, H-16β, -18α), 2.48 (1H, m, H-11α), 2.40 (1H, m, H-11β), 2.18 (1H, m, H-16α), 2.07 (2H, m, H-21α, -22β), 1.98 (1H, m, H-7β), 1.96 (1H, m, H-9α), 1.77 (1H, m, H-21β), 1.61 (1H, m, H-22α), 1.56 (2H, m, H_2-6), 1.42 (3H, m, H-7α, -12β, -19α), 1.40 (3H, s, H_3-25), 1.29 (1H, m, H-12α), 1.24 (3H, s, H_3-26), 1.23 (1H, m, H-19β), 1.22 (3H, s, H_3-27), 1.16 (3H, s, H_3-24), 1.10 (1H, m, H-5α), 1.07 (3H, s, H_3-30), 1.00 (3H, s, H_3-23), 0.99 (3H, s, H_3-29); ^{13}C-nmr data (125 MHz), see Table 1; negative-ion hrfabms *m/z* 727.3846 ($[M-H]^-$, 26), calcd for $C_{44}H_{55}O_9$, 727.3814.

*2*α-*Hydroxyaleuritolic acid 3*-p-*hydroxybenzoate* [**2**].—Amorphous solid; mp 280–281°; $[\alpha]_D$ −20.0° (*c*=0.2, pyridine); uv λ max (MeOH) (log ε) 256 (3.86) nm; ir ν max (KBr) 3400, 1690, 1608, 1275, 1160 cm^{-1}; ^1H nmr (C_5D_5N, 500 MHz) δ 8.30 (2H, d, *J*=8.5 Hz, H-2′, -6′), 7.18 (2H, d, *J*=8.5 Hz, H-3′, H-5′), 5.81 (1H, br d, *J*=5 Hz, H-15), 5.27 (1H, d, *J*=10 Hz, H-3α), 4.32 (1H, dt, *J*=4 and 10 Hz, H-2β), 2.81 (2H, m, H-16β, -18α), 2.29 (1H, dd, *J*=13 and 4 Hz, H-1β), 2.13 (1H, m, H-16α), 2.06 (1H, m, H-22β), 1.97 (1H, m, H-21α), 1.95 (1H, m, H-7β), 1.74 (1H, m, H-11α), 1.68 (1H, m, H-21β), 1.60 (1H, m, H-9α), 1.59 (2H, m, H-11β, -22α), 1.45 (1H, m, H-6α), 1.39 (2H, m, H-12β, -19α), 1.36 (1H, m, H-6β), 1.33 (1H, m, H-1α), 1.32 (1H, m, H-7α), 1.27 (1H, m, H-12α), 1.22 (1H, m, H-19β), 1.15 (3H, s, H_3-26), 1.08 (3H, s, H_3-30), 1.07 (3H, s, H_3-27), 1.06 (3H, s, H_3-24), 1.04 (1H, m, H-5α), 1.01 (3H, s, H_3-25), 1.00 (3H, s, H_3-29), 0.98 (3H, s, H_3-23); ^{13}C-nmr data (125 MHz), see Table 1; negative-ion hrfabms *m/z* 591.3685 ($[M-H]^-$, 100), calcd for $C_{37}H_{51}O_6$, 591.3663.

*1*β-*Hydroxyaleuritolic acid 3*-p-*hydroxybenzoate* [**3**].—Amorphous solid; mp 266–267°; $[\alpha]_D$ +7.8° (*c*=0.10, pyridine); uv λ max (MeOH) (log ε) 256.5 (4.30) nm; ir ν max (KBr) 3400, 1690, 1620, 1277, 1168 cm^{-1}; ^1H nmr (C_5D_5N, 500 MHz) δ 8.21 (2H, d, *J*=8.7 Hz, H-2′, -6′), 7.17 (2H, d, *J*=8.7 Hz, H-3′, -5′), 5.81 (1H, dd, *J*=8 and 3 Hz, H-15), 4.97 (1H, dd, *J*=12 and 4 Hz, H-3α), 3.65 (1H, dd, *J*=11.5 and 4 Hz, H-1α), 2.77 (2H, m, H-16β, -18α), 2.41 (1H, m, H-2α), 2.33 (2H, m, H_2-11), 2.23 (1H, m, H-2β), 2.12 (1H, m, H-16α), 2.02 (1H, m, H-22β), 2.00 (1H, m, H-12β), 1.92 (1H, m, H-7β), 1.77 (1H, m, H-9α), 1.74 (1H, m, H-12α), 1.54 (1H, m, H-22α), 1.49 (2H, m, H_2-6), 1.37 (1H, m, H-19α), 1.35 (1H, m, H-7α), 1.22 (2H, m, H_2-21), 1.20 (1H, m, H-19β), 1.20 (3H, s, H_3-26), 1.19 (3H, s, H_3-25), 1.14 (3H, s, H_3-27), 1.03 (3H, s, H_3-24), 1.01 (3H, s, H_3-30), 0.94 (3H, s, H_3-29), 0.90 (3H, s, H_3-23), 0.77 (1H, m, H-5); ^{13}C-nmr data (125 MHz), see Table 1; eims *m/z* 592 ($[M]^-$, 0.6), 575 (1), 574 (4), 548 (6), 546 (4), 454 (1), 436 (12), 359 (3), 358 (1), 248 (16), 234 (29), 203 (23), 189 (28), 121 (100); hreims *m/z* $[M]^+$ 592.3766, calcd for $C_{37}H_{52}O_6$, 592.3764.

*2*α-*Hydroxyaleuritolic acid 2,3*-bis-p-*hydroxybenzoate* [**4**].—Amorphous solid; mp 275–276°; $[\alpha]_D$ −38.6° (*c*=0.13, pyridine); uv, ir, and hrms were identical with those of previously reported data (3); ^1H nmr (C_5D_5N, 500 MHz) δ 8.26 and 8.25 (each 2H, d, *J*=8.7 Hz, H-2′, -6′, and H-2″, -6″), 7.08 (4H, d, *J*=8.7 Hz, H-3′, -5′, and H-3″, -5″), 5.82 (1H, m, H-15), 5.79 (1H, dd, *J*=10.5 and 4.4 Hz, H-2β), 5.50

(1H, d, J=10.5 Hz, H-3α), 2.81 (2H, m, H-16β, -18α), 2.33 (1H, dd, J=12.4 and 4.4 Hz, H-1β), 2.16 (1H, dd, J=14 and 3 Hz, H-16α), 1.26 (1H, m, H-1α), 1.16 (3H, s, H_3-27), 1.14 (3H, s, H_3-26), 1.12 (3H, s, H_3-25), 1.09 (3H, s, H_3-24), 1.07 (3H, s, H_3-30), 1.01 (3H, s, H_3-23), 1.00 (3H, s, H_3-29); ^{13}C-nmr data (125 MHz), see Table 1.

Aleuritolic acid 3-p-*hydroxybenzoate* [**5**].—Amorphous solid; mp, [α]D, uv, ir, and ms are identical with previously isolated maprounic acid 3-*p*-hydroxybenzoate (3); ^{1}H nmr (C_5D_5N, 250 MHz) δ 8.30 (2H, d, J=8.7 Hz, H-2′, -6′), 7.26 (2H, d, J=8.7 Hz, H-3′, -5′), 5.81 (1H, br d, J=5 Hz, H-15), 4.89 (1H, dd, J=11 and 5 Hz, H-3α), 2.80 (2H, m, H-16β, -18α), 1.15 (3H, s, H_3-26), 1.11 (3H, s, H_3-27), 1.10 (3H, s, H_3-30), 1.02 (6H, s, H_3-24, -29), 0.94 (3H, s, H_3-23), 0.88 (3H, s, H_3-25); ^{13}C-nmr data (62.5 MHz), see Table 1.

Aleuritolic acid [**6**].—Amorphous solid; mp, [α]D, ir, and ms are identical with previously reported data (3); ^{1}H nmr (C_5D_5N, 500 MHz) δ 5.81 (1H, dd, J=8 and 3 Hz, H-15), 3.41 (1H, dd, J=10 and 6 Hz, H-3α), 2.78 (2H, m, H-16β, -18α), 2.13 (1H, dd, J=14 and 3 Hz, H-16α), 1.19 (3H, s, H_3-23), 1.16 (3H, s, H_3-26), 1.09 (3H, s, H_3-27), 1.08 (3H, s, H_3-30), 1.01 (3H, s, H_3-24), 1.00 (3H, s, H_3-29), 0.90 (3H, s, H_3-25); ^{13}C-nmr data (125 MHz), see Table 1.

Aleuritolic acid 3-acetate [**7**].—Recrystallized from *n*-hexane/EtOAc; mp, [α]D, ir, ^{1}H- and ^{13}C-nmr data ($CDCl_3$) are identical with those reported for 3-acetylaleuritolic acid (12,19); ^{13}C-nmr data (C_5D_5N, 62.5 MHz), see Table 1.

BASIC HYDROLYSIS OF **3**.—Compound **3** (20 mg) was refluxed overnight with 6% KOH in H_2O. After cooling, the solution was carefully acidified to pH 3–4 with 30% H_2SO_4, then filtered and washed with H_2O. The precipitate was purified using a Si gel column, eluting with $CHCl_3$ and $(Me)_2CO$ (10:1). In fractions 11 through 14, 1β-hydroxyaleuritolic acid [**8**] (10 mg) was obtained: mp 280–283° (dec); [α]D +17.8° (c=0.05, MeOH); uv λ max (MeOH) (log ε) 212 (3.44) nm; ir ν max (KBr) 3408, 2926, 2859, 1688, 1466, 1400, 1298, 1252, 1001 cm^{-1}; ^{1}H nmr (C_5D_5N, 300 MHz) δ 5.90 (1H, dd, J=8.0 and 3.3 Hz, H-15), 3.71 (1H, dd, J=10.7 and 4.7 Hz, H-1α), 3.59 (1H, dd, J=11.6 and 4.7 Hz, H-3α), 2.85 (2H, m), 1.29 (3H, s), 1.27 (3H, s), 1.23 (3H, s), 1.21 (3H, s), 1.10 (3H, s), 1.08 (3H, s), 1.00 (3H, s); ^{13}C-nmr data (75.6 MHz), see Table 1; eims *m/z* 472 ([M]$^+$, 2.4), 454 (7), 426 (15), 318 (10), 248 (19), 234 (100), 189 (70), 119 (31).

BASIC HYDROLYSIS OF **4**.—Compound **4** (20 mg) was hydrolyzed by the same procedure as compound **3**. 2α-Hydroxyaleuritolic acid (sebiferenic acid) [**11**] (8 mg) was obtained: mp 297–299° (dec); [α]D −24.6° (c=0.15, MeOH); [lit. (20), mp 329° (dec), [α]D −32°]; uv λ max (MeOH) (log ε) 211 (3.82) nm; ir ν max (KBr) 3430, 2932, 2868, 1690, 1472, 1385, 1310, 1252 cm^{-1}; ^{1}H nmr (C_5D_5N, 300 MHz) δ 5.83 (1H, m, H-15), 4.15 (1H, m, H-2β), 3.39 (1H, d, J=9.5 Hz, H-3α), 2.80 (2H, m), 1.25 (3H, s), 1.18 (3H, s), 1.10 (6H, s), 1.09 (3H, s), 1.02 (3H, s), 1.01 (3H, s); ^{13}C-nmr data (75.6 MHz), see Table 1; fabms *m/z* 471 ([M−H]$^-$, 7), 455 (7), 437 (8), 299 (10), 223 (8), 207 (67), 187 (19), 115 (100).

METHYLATION OF **3**.—Compound **3** (10 mg) was dissolved in MeOH (20 ml) and CH_2N_2 was added in $(Et)_2O$ at room temperature. Needle crystals of methyl 1β-hydroxyaleuritolic acid 3-*p*-methoxybenzoate [**9**] (7 mg) were obtained by filtration: mp 261–262°; [α]D +34.0° (c=0.10, $CDCl_3$); uv λ max ($CHCl_3$) (log ε) 257.5 (4.47) nm; ir ν max (KBr) 2980, 2847, 1727, 1614, 1443, 1405, 1272, 1177, 1016 cm^{-1}; ^{1}H nmr ($CDCl_3$, 300 MHz) δ 7.93 (2H, d, J=8.8 Hz, H-2′, -6′) 6.84 (2H, d, J=8.8 Hz, H-3′, -5′), 5.47 (1H, dd, J=7.8 and 2.9 Hz, H-15), 4.64 (1H, dd, J=12.2 and 4.3 Hz, H-3α), 3.79 (3H, s, MeO-4′), 3.52 (3H, s, COOMe), 2.35 (2H, m), 1.20 (3H, s), 1.18 (3H, s), 1.00 (3H, s), 0.95 (3H, s), 0.92 (3H, s), 0.89 (3H, s), 0.84 (3H, s); ^{13}C-nmr data (75.6 MHz), see Table 1; eims *m/z* 620 ([M]$^+$, 7), 560 (11), 468 (22), 344 (16), 300 (15), 248 (45), 203 (19), 189 (56), 135 (100).

METHYLATION OF **4**.—Compound **4** (10 mg) was methylated by the same method as **3**. Methyl 2α-hydroxyaleuritolate 2,3-*bis*-*p*-methoxybenzoate [**12**] (8 mg) was obtained as needle crystals: mp 272–273°; [α]D −37.5° (c=0.30, $CDCl_3$); uv λ max (MeOH) (log ε) 257 (4.70) nm; ir ν max (KBr) 2969, 2866, 1728, 1609, 1510, 1451, 1283, 1167, 1028 cm^{-1}; ^{1}H nmr ($CDCl_3$, 300 MHz) δ 7.88 and 7.82 (each 2H, d, J=9 Hz, H-2′, -6′, and H-2″, -6″), 6.80 and 6.77 (each 2H, d, J=9 Hz, H-3′, -5′, and H-3″, -5″), 5.50 (1H, dd, J=8.0 and 3.7 Hz, H-15), 5.37 (1H dd, J=12.2 and 5.1 Hz, H-2β), 5.12 (1H, dd, J=10.5 Hz, H-3α), 3.78 and 3.77 (each 3H, s, MeO-4′ and -4″), 3.56 (3H, s, COOMe), 2.35 (2H, m), 1.23 (3H, s), 1.15 (3H, s), 1.06 (3H, s), 0.95 (6H, s), 0.91 (3H, s), 0.90 (3H, s); ^{13}C-nmr data (75.6 MHz), see Table 1; eims *m/z* 754 ([M]$^+$, 2.3), 694 (4), 602 (4), 507 (3), 450 (9), 435 (6), 391 (3), 262 (6), 248 (12), 203 (16), 189 (27), 135 (100).

ACETYLATION OF **3**.—Compound **3** (10 mg) was dissolved in pyridine (0.5 ml) and $(Ac)_2O$ (0.5 ml) was added at room temperature and allowed to stand overnight, and then poured into ice water and filtered. The precipitate purified with prep. tlc ($CHCl_3$-MeOH 50:1), 1β-acetoxyaleuritolic acid 3-*p*-acetoxybenzoate

[**10**] (8 mg), was obtained as white crystals: mp 258–259°; $[\alpha]_D$ +7.1° (c=0.14, $CHCl_3$); uv λ max ($CHCl_3$) (log ε) 247 (4.03) nm; ir ν max (KBr) 2947, 2862, 1730, 1694, 1602, 1472, 1392, 1273, 1209, 1163, 1115, 1017 cm^{-1}; ^1H nmr ($CDCl_3$, 300 MHz) δ 8.04 (2H, d, J=8.7 Hz, H-2′, -6′), 7.14 (2H, d, J=8.7 Hz, H-3′, -5′), 5.55 (1H, dd, J=7.5 Hz, H-15), 4.81 (1H, dd, J=12.5 and 4.6 Hz, H-3α), 4.73 (1H, dd, J=11.6 and 4.5 Hz, H-1α), 2.35 (1H, m), 2.32 (3H, s, AcO-4′), 2.00 (3H, s, AcO-1β), 1.25 (3H, s), 1.16 (3H, s), 1.03 (3H, s), 0.99 (3H, s), 0.94 (6H, s), 0.91 (3H, s); ^{13}C-nmr data (75.6 MHz), see Table 1; eims *m/z* 676 ($[M]^+$, 1), 633 ($[M-Ac]^+$, 0.6), 630 (5), 616 ($[M-OAc]^+$, 3), 523 (2), 436 (17), 235 (18), 234 (70), 190 (22), 189 (57), 163 (31), 133 (27), 121 (100).

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