# Maprouneacin, a New Daphnane Diterpenoid with Potent Antihyperglycemic Activity from *Maprounea africana*

John R. Carney,*,† Joann M. Krenisky,† R. Thomas Williamson,‡ Jian Luo,† Thomas J. Carlson,† Victor L. Hsu,§ and Joseph L. Moswa⊥

*Shaman Pharmaceuticals, Inc., 213 E. Grand Ave., South San Francisco, California, 94080, Departments of Chemistry and Biochemistry and Biophysics, Oregon State University, Corvallis, Oregon, 97331, and Faculté de Pharmacie, Université de Kinshasa, Democratic Republic of Congo*

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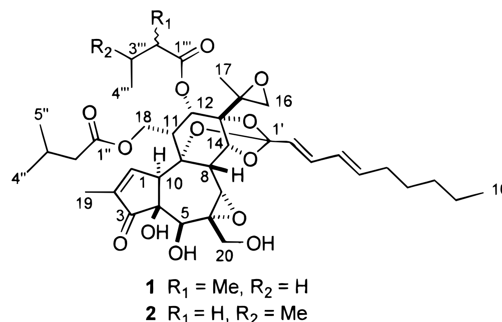
Bioassay-guided fractionation of the EtOH extract of *M. africana,* using the in vivo noninsulin-dependent diabetes mellitus *db*/*db* mouse model, resulted in the isolation of the new daphnane-type diterpenoid maprouneacin (**2**). Compound **2** showed potent glucose-lowering properties when given by the oral route.

*Maprounea africana* Muell. Arg. (Euphorbiaceae) has received considerable attention from chemists due to its extensive use in Central Africa in the treatment of a number of ailments.[1−5] As a result, many cytotoxic pentacyclic triterpenoids[5−8] and three diterpenoids—bershacolone[9] and koumbalones A and B[10]—have been described from this plant. Several of the triterpenoids isolated from *M. africana* were reported to have potent inhibitory activity against HIV-1 reverse transcriptase,[6] but during subsequent studies it was suggested that the activity originally ascribed to the triterpenoids may have been due to the presence of trace amounts of a daphnane-type diterpenoid.[5] Unfortunately, not enough compound was available for full characterization, but the authors noted a highly oxidized resiniferonol derivative (**1**) had been reported from *Maprounea membranacea* in a Japanese patent.[5,11]

We investigated *M. africana* as part of our ethnobotanical-directed approach to the discovery of compounds for the treatment of type 2 or noninsulin-dependent diabetes mellitus (NIDDM). Reports in the literature[2] and by healers in the Republic of Congo of the use of decoctions of *M. africana* in the treatment of NIDDM were substantiated by potent antihyperglycemic activity of the extract in our in-house NIDDM model using genetically diabetic *db*/*db* mice.[12] Bioassay-directed fractionation of the aqueous EtOH extract of *M. africana* by liquid−liquid partitioning, followed by LH-20 chromatography, gave a fraction with potent antihyperglycemic activity. Further purification by ODS solid-phase extraction cartridges and HPLC provided small amounts of maprouneacin (**2**) as an unstable, colorless glass.

The HRFABMS data indicated a molecular formula of $C_{40}H_{56}O_{13}$ for **2**, which is isomeric with **1**. The $^1$H NMR shifts for the diterpenoid portion of **2** were in excellent agreement with those reported for **1**.[11] $^{13}$C NMR shifts for **1** were not reported and were assigned for **2** from HMQC and HMBC NMR data (Table 1). The chemical shifts for H-2′ through $H_3$-10′ and $H_2$-2″ through $H_3$-5″ of **2** were also in agreement with those reported for **1**. Complete analysis of the $^1$H, $^{13}$C, COSY, HMQC, and HMBC data (Table 1) fully supported the structural similarities of **2** and **1** and




revealed that they differed in the ester at C-12. The carbon shifts eventually assigned to C-1″ through C-5″ were comparable to those for C-1‴ through C-5‴, suggesting the presence of two isovalerate groups in **2**. Specifically, an HMBC correlation from H-12 to a carbon signal at δ 172.1 (C-1‴) linked an ester to C-12. HMBC correlations were also observed to C-1‴ from two protons at δ 2.15, both attached to a carbon resonating at δ 43.2 (C-2‴), and from one at δ 2.00, bound to a carbon with a signal at δ 25.6 (C-3‴). Of the three methyl signals at δ 0.89 in the $^1$H NMR spectrum of **2**, one was assigned to the terminus of the ortho ester, and it showed the expected two- and three-bond HMBC correlations to C-8′ and C-9′. The remaining two methyl signals displayed long-range couplings to C-2‴ and C-3‴ and COSY correlations to H-3‴. HMBC cross-peaks from $H_2$-2‴ and H-3‴ to C4‴/C5‴ were also observed. The chemical shifts for the isovalerate moieties of **2** are in good agreement with published values,[13] although the $^1$H NMR shifts reported for the α-methylbutyrate moiety in **1**—δ 2.0 (1H, m), 1.2−1.3 (2H), and 0.9 (6H, m)—do not appear to be consistent with the structure.[13] Without $^{13}$C NMR data for **1** or a sample for direct comparison, it is difficult to conclude whether the structure of **1** should be revised to **2**, or if the $^1$H NMR shifts were misassigned. We planned to isolate **1** from an extract of *M. membranacea* from our repository for comparison, but, assuming **1** and **2** would have similar retention times, neither could be detected by photodiode array-detected HPLC analysis. Compound **2** was readily detectable in a lipophilic extract of *M. africana* under the same analysis conditions.

A coupling constant of 8.0 Hz between H-11 and H-12 in **1** and **2**, in contrast to a value of 0−1 Hz commonly observed for these protons in other daphnanes functionalized at C-12, suggested that **2** has the same unusual

* To whom correspondence should be addressed. Tel: (650) 952-7070. Fax: (650) 873-8377. E-mail: jcarney@shaman.com.
† Shaman Pharmaceuticals.
‡ Department of Chemistry, Oregon State University.
§ Department of Biochemistry and Biophysics, Oregon State University.
⊥ Université de Kinshasa.

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**Table 1.** NMR Data Reported for **1**[11] and for **2** (all in $CDCl_3$)

| | **1** | | **2** | |
|---|---|---|---|---|
| position | $^1$H (400 MHz) | $^1$H (600 MHz) | $^{13}$C (100 MHz) | HMBC |
| 1 | 7.64 (br s) | 7.64 (br s) | 158.7 | C-2, C-3, C-4, C-10, C-19 |
| 2 | | | 138.5 | |
| 3 | | | 208.8 | |
| 4 | | | 72.5 | |
| OH-4 | | 3.64 (s) | | C-4, C-10 |
| 5 | 4.26 (s) | 4.27 (s) | 71.5 | C-3, C-4, C-6, C-7 |
| OH-5 | | 4.00 (s) | | C-5(w),[a] C-6 |
| 6 | | | 60.7 | |
| 7 | 3.47 (br s) | 3.47 (br s) | 62.9 | C-6, C-9 and/or C-14, C-20 |
| 8 | 3.24 (d, 2.6) | 3.24 (d, 2.5) | 34.4 | C-6, C-7, C-9 and/or C-14 |
| 9 | | | 79.4 | |
| 10 | 3.80 (br s) | 3.80 (d, 2.5) | 47.7 | |
| 11 | 2.88 (m) | 2.90 (t, 8.0) | 45.0 | C-9, C-10, C-12, C-13, C-18 |
| 12 | 5.44 (d, 7.6) | 5.44 (d, 8.0) | 69.5 | C-11, C-13, C-14, C-18, C-1‴ |
| 13 | | | 83.2 | |
| 14 | 4.60 (d, 2.6) | 4.60 (d, 2.5) | 79.3 | C-7, C-9, C-15, C-1′ |
| 15 | | | 56.4 | |
| 16 | 2.49 (d, 4.8) | 2.49 (d, 5.0) | 48.3 | C-13, C-15 |
| | 2.75 (d, 4.8) | 2.75 (d, 5.0) | | C-13, C-15 |
| 17 | 1.37 (s) | 1.36 (s) | 18.2 | C-13, C-15, C-16 |
| 18 | 4.39 (dd, 13.1, 5.5) | 4.39 (dd, 13.0, 7.0) | 60.6 | C-1″, C-9 (w), C-11, C-12 |
| | 4.76 (d, 13.1) | 4.76 (d, 13.0) | | C-1″, C-9, C-11, C-12, |
| 19 | 1.75 (br s) | 1.76 (br s) | 9.8 | C-1, C-2, C-3 |
| 20 | 3.79 (d, 12.6) | 3.78 (d, 12.5) | 64.6 | C-5 (w), C-6, C-7 |
| | 3.91 (d, 12.6) | 3.91 (d, 12.5) | | C-6, C-7 |
| 1′ | | | 118.1 | |
| 2′ | 5.59 (d, 15.5)[b] | 5.59 (d, 15.5) | 120.8 | C-1′, C-3′, C-4′ |
| 3′ | 6.64 (dd, 15.5, 10.6) | 6.64 (dd, 15.5 10.5) | 135.5 | C-1′, C-2′, C-4′, C-5′ |
| 4′ | 6.02 (dd, 15.3, 10.6) | 6.02 (dd, 15.5, 10.5) | 128.1 | C-2′, C-3′, C-5′, C-6′ |
| 5′ | 5.88 (dt, 15.3, 7.3) | 5.88 (dt, 15.0, 8.0) | 140.0 | C-3′, C-4′, C-6′, C-7′ |
| 6′ | 2.10 (m) | 2.10 (q) | 32.6 | C-4′, C-5′, C-7′, C-8′ |
| 7′ | 1.2−1.4 | 1.40 (m) | 28.7 | C-5′, C-6′, C-8′, C-9′ |
| 8′ | 1.2−1.4 | 1.28 (m) | 31.3 | |
| 9′ | 1.2−1.4 | 1.24 (m) | 22.3 | |
| 10′ | 0.90 | 0.89 (m) | 14.0 | C-8′, C-9′ |
| 1″ | | | 172.3 | |
| 2″ | 2.38 (dd, 6.6, 4.8) | 2.37 (dd, 15.0, 6.5) | 43.2 | C-1″, C-3″, C-4″, C-5″ |
| | 2.35 (m) | 2.23 (m) | | C-1″, C-3″, C-4″, C-5″ |
| 3″ | 2.20 (m) | 2.18 (m) | 25.2 | |
| 4″ | 0.98 (d, 6.7) | 0.98 (d) | 22.4 | C-2″, C-3″, C-5″ |
| 5″ | 0.97 (d, 6.7) | 0.98 (d) | 22.4 | C-2″, C-3″, C-4″ |
| 1‴ | | | 172.1 | |
| 2‴ | 2.0 (m) | 2.15 (m) | 43.2 | C-1‴ |
| 3‴ | 1.2−1.3 (m) | 2.00 (m) | 25.6 | C-1‴ |
| 4‴ | 0.90 (m) | 0.89 (m) | 22.3 | C-2‴, C-3‴, C-5‴ |
| 5‴ | 0.90 (m) | 0.89 (m) | 22.3 | C-2‴, C-3‴, C-4‴ |

[a] Weak correlation. [b] All shifts for the nonterpenoid portion of **1** were not specifically assigned, but are presented for comparison.

**Table 2.** Changes in Glucose Levels, Body Weight, and Food Consumption for Vehicle, Positive Control, and **2**

| | change in plasma glucose levels, pre-dose vs post-dose (mg/dL) | | body weight, average (g/mouse) | | food intake (g/mouse) |
|---|---|---|---|---|---|
| treatment | 3 h | 27 h | 0 h | 24 h | average |
| vehicle | −28 | −27 | 40.2 ± 0.5 | 40.1 ± 0.4 | 6.7 |
| metformin[a] | −132 | −160 | 41.0 ± 0.8 | 40.9 ± 0.8 | 7.3 |
| maprouneacin (**2**)[b] | −254 | −246 | 37.7 ± 0.7 | 37.8 ± 0.8 | 0.1 |

[a] Single dose, 250 mg/kg. [b] Single dose, 0.5 mg/kg.

relative stereochemistry at C-12 as found in **1**. Double pulsed-field-gradient spin-echo (DPFGSE) NOE experiments[14] were used to confirm this. The ortho ester requires that the six-membered ring be in a twisted-boat conformation, placing H-12 in close proximity to H-8. Indeed, irradiation of H-8 enhanced the signals of H-11, H-12, H-7, and H-14. Additional NOE data, obtained by irradiation of H-14, which enhanced the signals of H-7, H-8, and H-17, and irradiation of H-5, which enhanced the H-10 signal, and comparison of chemical shifts for the diterpenoid skeleton to published values,[15] are consistent with the proposed relative stereochemistry. To the best of our knowledge, **1** and **2** are the first daphnanes to have this stereochemistry at C-12.

When dosed at 0.5 mg/kg qd, **2** lowered serum glucose levels below those in our positive control group treated with metformin, a drug clinically used for the treatment of NIDDM, dosed at 250 mg/kg qd (Table 2). Because a dramatic decrease in glucose levels was observed at 3 h, the glucose-lowering effect observed for **2** cannot be due solely to the lack of food consumption during the test period.

Healers in the Republic of Congo prepare a decoction of *M. africana* and *Crossopteryx febrifuga* by extracting the

roots of both simultaneously for 30 min to treat middle-aged men and women with increased urination in the day and night, which is a symptom of NIDDM. The extract (25−30 mL) is taken orally 2−3 times per day until symptoms disappear. We prepared an aqueous extract of *M. africana* by boiling the root material in water, filtering it, and treating it with 2-propanol. A $CH_2Cl_2$ extract of the clarified aqueous extract was analyzed by photodiode array-detected ODS HPLC. Although **2** is relatively lipophilic and its concentration in an aqueous extract would be expected to be low, it was readily detectable. These results suggest that **2** is responsible, at least in part, for the efficacy of this treatment by the healers.

The daphnanes, tiglianes, and ingenanes, which compose a large class of structurally related diterpenoids frequently referred to collectively as "phorbol esters,"[16] occur in plants of the Euphorbiaceae and Thymelaeaceae. Thirty species from these families have been associated with the treatment of diabetes by traditional healers.[17] Phorbol esters are infamous due to the pronounced irritant properties exhibited by most, and many are potent tumor promoters and activate protein kinase C (PKC). But because pro-inflammatory activity does not correlate directly to tumor-promoting activity,[16] several recent reports have indicated a regenerated interest in the bioactivities of phorbol esters.[18,19] Of perhaps greatest interest is a highly irritant daphnane, resiniferatoxin, which has a low affinity for PKC, is a potent vanilloid agonist, and has excellent therapeutic potential for treatment of neurogenic pain and inflammation.[20] A tyrosine kinase is involved in cell signaling at the insulin receptor, and it is tempting to attribute the dramatic glucose-lowering effect observed for **2** to a PKC-mediated response, but there are drastically conflicting reports on the nature of the relationship, if any, of PKC to insulin action.[21] Further biological studies with **2** and semi-synthetic derivatives are underway.

## Experimental Section

**General Experimental Procedures.** NMR spectra were recorded on Bruker DRX-600 and AM-400 spectrometers. A 60-ms Gaussian pulse width with 1K data points truncated at 3% was used for the DPFGSE experiments. MS were measured on a Kratos MS 50 TC spectrometer, and IR spectra were recorded on a Nicolet 510 spectrophotometer. A Hitachi system, composed of an L-6200A pump, AS-2000 autosampler, and L-4500A diode array detector, was used for HPLC. UV spectra were obtained from the diode array-detected HPLC.

**Plant Material.** Roots of *Maprounea africana* Muell. Arg. were collected in the Kinshasa region of the Republic of Congo (Zaire) in November 1996, and authenticated by Mary Merello of the Missouri Botanical Garden. A voucher specimen has been deposited in the reference collection, Department of Ethnobotany and Conservation, Shaman Pharmaceuticals, Inc., and at the herbarium of the Université de Kinshasa, Democratic Republic of Congo.

**Bioassay.** The NIDDM *db*/*db* mouse model bioassay used has been described in detail elsewhere.[12]

**Extraction and Isolation.** The air-dried, milled root material of *M. africana* (2.60 kg) was extracted with EtOH−$H_2O$, 8:2 (8.5 L) for 70 h at room temperature, and the residue (127 g) was partitioned between $CH_2Cl_2$−$H_2O$, 1:1 (4 L). The lower layer residue (29.4 g) was dissolved/suspended in EtOH−$H_2O$, 9:1 (2.1 L) and extracted twice with petroleum ether (1.5 L for the first extraction, 900 mL for the second). The EtOH-layer residue (20.4 g) was dissolved/suspended in MeOH−$H_2O$, 8:2 (500 mL), extracted with $CCl_4$ (2 × 500 mL), and the resulting lower-layer residue (14.7 g) was partitioned between hexanes and MeOH−$H_2O$, 9:1 (1.5 L). The lower-layer residue (9.7 g) was fractionated over Sephadex LH-20 (2.5 × 76.2 cm, 20 mL/fraction), eluting with $CH_2Cl_2$−MeOH (1:1). The sixth fraction past the column void volume (428 mg) had the greatest glucose-lowering effect and was further fractionated. Three 60-mg subsamples of the residue were each passed through 6-mL ODS Bond-Elut solid-phase extraction cartridges (Varian), eluting with MeCN−$H_2O$ (6:4), to yield a total of 90 mg. Final purification by HPLC (YMC ODS-AQ, 4.6 × 250 mm, MeCN−$H_2O$, 68:32, 1.7 mL/min) gave 4 mg of **2**. These HPLC conditions were also used to assay for **2** in lipophilic extracts of *M. africana* and *M. membranacea* and the aqueous extract of *M. africana*.

**Maprouneacin (2):** colorless, unstable glass; UV (MeCN−$H_2O$, 6:4) $\lambda_{max}$ 235 nm; IR (thin film) $\nu_{max}$ 2960, 1731, 1711, 1094 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR, see Table 1; FABMS (positive ion, oxalic acid/thioglycerol/glycerol) *m*/*z* 767 [M+Na], 745 [M + H], 588, 566, 85; HRFABMS *m*/*z* 745.3795 (calcd for $C_{40}H_{57}O_{13}$, 745.3799).

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**Supporting Information Available:** $^1H$ NMR, $^{13}C$ NMR, and DPGSE NOE spectra for maprouneacin (**2**) (7 pages). Ordering information is given on any current masthead page.

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