Pentacyclic Triterpenes Derived from Maprounea africana Are Potent Inhibitors of HIV-1 Reverse Transcriptase

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PENTACYCLIC TRITERPENES DERIVED FROM *MAPROUNEA AFRICANA* ARE POTENT INHIBITORS OF HIV-1 REVERSE TRANSCRIPTASE

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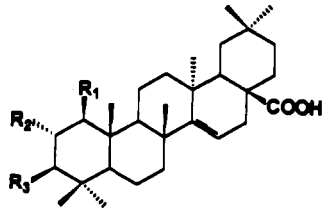
ABSTRACT.—Two pentacyclic triterpenes isolated from *Maprounea africana*, 1β-hydroxymaprounic acid 3-*p*-hydroxybenzoate [**3**], 2α-hydroxymaprounic acid 2,3-*bis*-*p*-hydroxybenzoate [**4**] and their respective hydrolyzed products [**5**] and [**6**], have been found to demonstrate potent inhibitory activity against HIV-1 reverse transcriptase.

Over the past decade, substantial progress has been made in defining strategies for the treatment of human immunodeficiency virus (HIV) infection, the cause of acquired immunodeficiency syndrome (AIDS). Because reverse transcriptase (RT) is required for early proviral DNA synthesis (1), inhibition of the RT-catalyzed polymerization of DNA from viral RNA inhibits virus replication. RTs may be viral-specific and are thus considered viable chemotherapeutic targets. Most potent HIV-RT inhibitors are nucleoside analogs that are converted to triphosphates by cellular enzymes and act as chain terminators (2,3). As examples, the only drugs approved for use in HIV-1 infection to date are azidothymidine (AZT), didanosine (dideoxyinosine or ddI), and zalcitabine (dideoxycytidine or ddC) (4). Although these compounds have been shown to benefit HIV-infected individuals, there are toxic side-effects associated with their use (4), and complete inhibition of viral replication is rarely achieved (4–6). In addition, the emergence of nucleotide-resistant HIV strains may complicate long-term therapy (7).

Most reverse-transcriptase inhibitors [such as antimonitungstate (8) and suramin (9)] also inhibit cellular DNA or RNA polymerases, and the nonselectivity of such agents can contribute to in vivo toxicity (10,11). Hence, the discovery and characterization of agents capable of specifically inhibiting HIV RT without mediating a toxic response remains a high priority. Natural products serve as one source of structurally novel chemicals that are worth investigating as specific inhibitors of HIV RT. Previous screening of various natural products for HIV-1 RT inhibitory activity showed stringent structural requirements (12), since few compounds demonstrated potent activity. Natural product RT inhibitors, such as benzophenanthridine (13) and protoberberine (14) alkaloids, flavonoids (15,16), a variety of other compounds with phenolic hydroxy groups (17,18), and certain antibiotics (19), were found to inhibit HIV-1 RT (as well as HIV-2 RT) with similar potency (12). We have also shown that *O*-methylpsychotrine is a selective inhibitor of HIV-1 RT (20) and even greater activity was observed with HIV-2 RT and this compound (21).

In our continuing efforts in this area of research, an extract derived from the roots of *Maprounea africana* Muell.-Arg. (Euphorbiaceae) collected in Tanzania, yielded four pentacyclic triterpenes: maprounic acid [**1**], maprounic acid acetate [**2**], 1β-hydroxymaprounic 3-*p*-



1 $R_1=R_2=H$, $R_3=OH$

2 $R_1=R_2=H$, $R_3=O-C(=O)-CH_3$

3 $R_1=OH$, $R_2=H$, $R_3=O-C(=O)-C_6H_4-OH$

4 $R_1=H$, $R_2=R_3=O-C(=O)-C_6H_4-OH$

5 $R_1=R_3=OH$, $R_2=H$

6 $R_1=H$, $R_2=R_3=OH$

hydroxybenzoate [**3**], and 2α-hydroxymaprounic acid 2,3-*bis*-*p*-hydroxybenzoate [**4**]. We currently report the potential of these four compounds to inhibit RT, as well as **5** and **6**, the hydrolyzed products of **3** and **4**. Compounds **3–6** were found to inhibit HIV-1 RT with IC_{50} values in the range of 3–5 μM (Table 1). Under these reaction conditions, the most potent inhibitory activity we have previously observed with plant secondary metabolites have been mediated by fagaronine (IC_{50}=13 μM) (12) and *O*-methylpsychotrine (IC_{50}=14 μM) (20). Thus, we consider compounds **3–6** potent inhibitors of reverse transcriptase. Compound **1** was approximately fivefold less active against HIV-1 RT, relative to compounds **3–6**, and the acetate of **1** (compound **2**) was approximately 35-fold less active. All six compounds were less active toward HIV-2 RT, relative to HIV-1 RT (Table 1). Compounds **3** and **4** and their hydrolyzed products (compounds **5** and **6**) all have similar HIV RT inhibitory potency. The mechanism of inhibition is currently under investigation.

TABLE 1. Inhibition of HIV-1 and HIV-2 RTs by Compounds **1–6**.[a]

Compound	HIV-1 RT	HIV-2 RT
1	24.9 (0.999)	78.7 (0.965)
2	136.0 (0.972)	>200
3	3.7 (0.983)	59.0 (0.989)
4	3.7 (0.996)	28.9 (0.927)
5	4.9 (0.997)	53.1 (0.988)
6	3.4 (0.999)	56.7 (0.916)

[a]IC_{50} values (μM) followed by the correlation coefficient obtained from the dose-response curve (in parentheses).

This report comprises the first example of pentacyclic triterpenes that mediate potent inhibition of HIV RT. From this limited series of six compounds, we can surmise that the greater activity demonstrated by compound **5**, relative to compound **1**, is due to the presence of two hydroxy groups. Furthermore, the ester groups of compounds **3** and **4** can be removed, without loss of activity, based on the fact that compounds **5** and **6** are equipotent as HIV-1 RT inhibitors. These data indicate that other types of structural modifications might be readily implemented without loss of activity. As a result of these features, compounds of this structural class are prime candidates for drug development.

EXPERIMENTAL

PLANT MATERIAL.—The roots of *Maprounea africana* were collected and identified in Tanzania by Mr. E.N. Mshiu. A voucher specimen has been retained in the herbarium of the PCRPS, College of Pharmacy, University of Illinois at Chicago.

COMPOUND ISOLATION AND IDENTIFICATION.—An EtOAc-soluble extract (32.2 g) obtained from an MeOH percolate of *M. africana* roots (2.5 kg), was purified by a combination of cc over Si gel and Sephadex LH-20 and prep. tlc. Two triterpene esters, **3** (1β-hydroxymaprounic acid 3-*p*-hydroxybenzoate; 140 mg, 0.012% w/w) and **4** (2α-hydroxyasprounic acid 2,3-*bis*-*p*-hydroxybenzoate; 100 mg, 0.016% w/w) were obtained as a result of this purification, and exhibited: **3**, mp 266–267°, [α]D +7.8° (*c*=0.103, C_5H_5N), hreims, *m/z* $[M]^+$ 592.3766, calcd for $C_{37}H_{52}O_6$ 592.3764; **4**, mp 275–6°, [α]D −38.6° (*c*=0.13, C_5H_5N), hrfabms, *m/z* $[M+H]^+$ 713.4061, calcd for $C_{44}H_{57}O_8$ 713.4053. Compounds **5** (1β-hydroxymaprounic acid) and **6** (2α-hydroxymaprounic acid) were produced by hydrolysis of **3** and **4**, respectively, with 6% KOH in H_2O (refluxed overnight), and exhibited: **5**, mp 280–283°, [α]D +4.9° (*c*=0.12, C_5H_5N), eims, *m/z* 472 $[M]^+$ and **6**, mp 297–299°, [α]D +25.0°

(c=0.18, C_5H_5N) [lit. (22) mp 325° (dec), $[\alpha]_D$ +32° ($CHCl_3$)]; fabms, *m/z* 471 $[M-H]^-$. Compounds **1** (maprounic acid) and **2** (maprounic acid acetate) were isolated from *M. africana* roots as described earlier (23,24).

The original EtOAc-soluble extract was found to demonstrate inhibitory activity against HIV-1 RT; the extract was also active as an inhibitor of phorbol 12,13-dibutyrate (PDBu) binding with partially purified protein kinase C. Thus, compounds **3** and **4** were isolated by activity-guided fractionation using the phorbol ester binding assay, essentially as described by Beutler *et al.* (25). The isolates were tested in the in vitro HIV-1 RT assay and found to demonstrate the activities described herein. Although compound **3** is a new compound, it has been necessary to reassign the structure of **4**. Full details of the characterization of these compounds and several analogs, as well as their evaluation in biological test systems germane to cancer chemoprevention, will be provided in a further publication.

REAGENTS AND TEMPLATES.—[^{3}H]TTP (15 Ci/mmol) was obtained from ICN Radiochemicals (Irvine, CA). TTP, poly(rA), oligo $(dT)_{12-18}$, dithiothreitol, and glutathione were purchased from Sigma Chemical Company (St. Louis, MO). DEAE-cellulose filter discs (Whatman DE 81) were obtained from VWR Scientific (Batavia, IL). All other reagents were of analytical grade.

HIV-1 (P66/P51) AND HIV-2 (P68/P55) REVERSE TRANSCRIPTASES (HIV-1 AND HIV-2 RTs).—Dimeric HIV-1 RT was purified by modification of the procedures of Clark *et al.* (26). HIV-2 RT is a recombinant enzyme consisting of two polypeptide subunits (68 and 55 kDa). It was synthesized in an *Escherichia coli* expression system using a genetically engineered plasmid (27). The enzyme possesses both RNA-dependent DNA polymerase and ribonuclease H activities typical of retroviral RTs.

HIV-1 AND HIV-2 RT ASSAYS.—Assays were conducted as described previously (12). Identical conditions were utilized for assays performed with HIV-1 and HIV-2 RTs (cf. 28). The assay mixture (final volume 100 μl) contained the following: 50 mM Tris·HCl buffer (pH 8.0), 150 mM KCl, 5 mM $MgCl_2$, 0.5 mM ethylene glyco-*bis*-(β-aminoethylether) *N,N'*-tetraacetic acid (EGTA), 5 mM dithiothreitol, 0.3 mM glutathione, 2.5 μg/ml bovine serum albumin, 41 μM poly(rA) [ϵ_{260} (mM)=7.8], 9.5 μM oligo $(dT)_{12-18}$ [ϵ_{260} (mM)=5.6], 20 μM TTP, and 0.5 μCi [^{3}H]TTP. The reaction was started by the addition of 10 μl (0.08 μg) of HIV-1 RT, and the mixture was permitted to incubate at 37° for 1 h. Reactions were terminated by the addition of 25 μl of 0.1 M EGTA followed by chilling on ice. Aliquots of each reaction mixture (100 μl) were then spotted uniformly onto circular 2.5 cm DE-81 (Whatman) filters, kept at ambient temperature for 15 min, and washed four times with 5% aqueous Na_2HPO_4. 7 H_2O. This was followed by two more washings with doubly-distilled H_2O. Finally, the filters were thoroughly dried and subjected to scintillation counting.

For testing enzyme inhibition, five serial dilutions of samples in DMSO (10 μl) were added to the reaction mixtures prior to the addition of enzyme. The median inhibitory concentration (IC_{50}) was calculated from a linearly regressed dose-response plot of percent control activity vs. concentration of compound, utilizing at least five concentrations of each compound. Each data-plot represents the average of duplicate tests.

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LITERATURE CITED

1. P.S. Sarin, *Annu. Rev. Pharmacol. Toxicol.*, **28**, 411 (1988).
2. L. Toji and S.S. Cohen, *Proc. Natl. Acad. Sci. USA*, **63**, 871 (1969).
3. H. Mitsuya, K.J. Weinhold, P.A. Furman, M.H. St. Clair, S. Nusinoff-Lehrman, R.C. Gallo, D. Bolognesi, D.W. Barry, and S. Broder, *Proc. Natl. Acad. Sci. USA*, **82**, 7096 (1985).
4. M.I. Johnson and D.F. Hoth, *Science*, **260**, 1286 (1993).
5. D.D. Richman, M.A. Fischl, M.H. Grieco, M.S. Gottlieb, P.A. Volberding, O.L. Laskin, J.M. Leedom, J.E. Groopman, D. Mildvan, M.S. Hirsch, G.G. Jackson, D.T. Durack, S. Nusinoff-Lehrman, and the AZT Collaborative Working Group, *N. Engl. J. Med.*, **317**, 192 (1987).
6. D.D. Ho, T. Moudgil, and M. Alam, *N. Engl. J. Med.*, **321**, 1622 (1989).
7. B.A. Larder, G. Darby, and D.D. Richman, *Science*, **243**, 1731 (1989).
8. D. Domont, B. Spire, F. Barré-Sinoussi, L. Montagnier, and J.C. Chermann, *Ann. Inst. Pasteur/Virol.*, **136E**, 75 (1985).
9. K.D. Jentsch, G. Hunsmann, H. Hartmann, and P. Nickel, *J. Gen. Virol.*, **68**, 2183–2192 (1987).

10. K. Ono, H. Nakane, F. Barré-Sinoussi, and J.C. Chermann, *Eur. J. Biochem.*, **176**, 305 (1988).
11. K. Ono, H. Nakane, and M. Fukushima, *Eur. J. Biochem.*, **172**, 349 (1988).
12. G.T. Tan, J.M. Pezzuto, A.D. Kinghorn, and S.H. Hughes, *J. Nat. Prod.*, **54**, 143 (1991).
13. M.L. Sethi, *J. Nat. Prod.*, **42**, 187 (1979).
14. M.L. Sethi, *J. Pharm. Sci.*, **72**, 538 (1983).
15. G. Spedding, A. Ratty, and E. Middleton, Jr., *Antiviral Res.*, **12**, 99 (1989).
16. K. Ono, H. Nakane, M. Fukushima, J.C. Chermann, and F. Barré-Sinoussi, *Eur. J. Biochem.*, **190**, 469 (1990).
17. N. Kakiuchi, M. Hattori, T. Namba, M. Nishizawa, T. Yamagishi, and T. Okuda, *J. Nat. Prod.*, **48**, 614 (1985).
18. H. Nakane, M. Arisawa, A. Fujita, S. Koshimura, and K. Ono, *FEBS Lett.*, **286**, 83 (1991).
19. M.A. Chirigos, J.W. Pearson, T.S. Papas, W.A. Woods, H.B. Wood, Jr., and G. Spahn, *Cancer Chemother. Res.*, **57**, 305 (1973).
20. G.T. Tan, A.D. Kinghorn, S.H. Hughes, and J.M. Pezzuto, *J. Biol. Chem.*, **266**, 23529 (1991).
21. G.T. Tan, J.F. Miller, A.D. Kinghorn, S.H. Hughes, and J.M. Pezzuto, *Biochem. Biophys. Res. Commun.*, **185**, 370 (1992).
22. B.P. Pradhan, S. De, A. Nath, and J.N. Shoolery, *Phytochemistry*, **23**, 2593 (1984).
23. M.C. Wani, J.P. Schaumberg, H.L. Taylor, J.B. Thomson, and M.E. Wall, *J. Nat. Prod.*, **46**, 537 (1983).
24. A.T. McPhail, D.R. McPhail, M.C. Wani, M.E. Wall, and A.W. Nicholas, *J. Nat. Prod.*, **52**, 212 (1989).
25. J.A. Beutler, A.B. Alvarado, T.G. McCloud, and G.M. Cragg, *Phytother. Res.*, **3**, 188 (1989).
26. P.K. Clark, A.L. Ferris, D.A. Miller, A. Hizi, K.-W. Kim, S.M. Deringer-Boyer, M.L. Mellini, A.D. Clark, Jr., G.F. Arnold, W.B. Lebherz III, E. Arnold, G.M. Muschik, and S.H. Hughes, *AIDS Res. Hum. Retroviruses*, **6**, 753 (1990).
27. A. Hizi, R. Tal, and S.H. Hughes, *Virology*, **180**, 339 (1991).
28. A. Hizi, R. Tal, M. Shaharabany, and S. Loya, *J. Biol. Chem.*, **266**, 6230 (1991).

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