Structure, Absolute Stereochemistry, and Synthesis of Conocurvone, a Potent, Novel HIV-Inhibitory Naphthoquinone Trimer from a *Conospermum* sp.[1]

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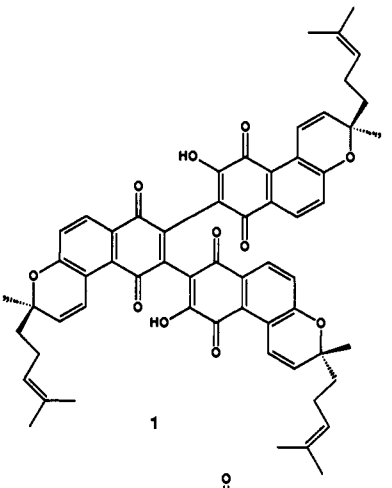
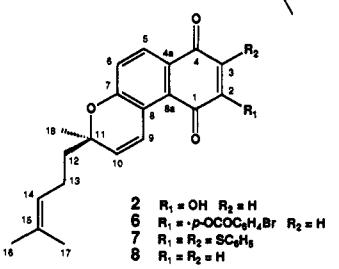
Abstract: Bioassay-guided fractionation provided conocurvone (**1**), a novel trimeric naphthoquinone derivative, as the active anti-HIV constituent of an extract from a *Conospermum* sp. The related naphthoquinone monomer teretifolione B (**2**) also was isolated from a *Conospermum* sp. extract. The absolute stereochemistry of **2** was established by X-ray crystallographic analysis of the *p*-bromobenzoate derivative **6**. Base-catalyzed coupling of 2 equiv of teretifolione B (**2**) with the deoxy derivative **8** provided compound **1**, which was identical in all respects with the natural product. While compound **2** was inactive against HIV, the natural and synthetic conocurvone (**1**) and the synthetic trimeric analog **4** were all active and equipotent, preventing the cytopathic effects and replication of HIV in human T-lymphoblastic cells (CEM-SS) over a concentration range of 0.02–50 μM.

As part of a major new natural products drug discovery and development initiative, the U.S. National Cancer Institute has been screening extracts from diverse terrestrial plants, marine organisms, and microbial sources for anti-HIV or selective cytotoxic properties.[2] The initial focus of the present study was an organic extract from the endemic Australian shrub *Conospermum* sp. (Proteaceae) (Spjut 7139), which inhibited the cytopathic effects of HIV-1 infection in the NCI's primary *in vitro* anti-HIV screen.[2,3] Bioassay-guided fractionation of the extract provided conocurvone (**1**), a novel trimeric naphthoquinone derivative, as the principal anti-HIV constituent. A solvent/solvent partitioning protocol applied to the crude extract concentrated HIV-inhibitory activity in the nonpolar fractions. This material was then subjected to centrifugal countercurrent chromatography (hexane/EtOH/EtOAc/H_2O, 5:4:2:1, ascending mode). Active fractions from the countercurrent chromatograph were pooled and further separated by low-pressure column chromatography on diol-bonded phase packing eluted with increasingly polar mixtures of hexane/EtOAc. Final purification of the anti-HIV constituent conocurvone (**1**) was achieved by HPLC on a phenyl-bonded phase column with CH_3CN/H_2O (17:3, 0.1% HOAc by vol).

During fractionation of a *Conospermum* sp. extract, a related monomeric species that was inactive against HIV was isolated and identified by spectral analysis as teretifolione B (**2**). This compound had previously been identified as one of a series of quinones from *C. teretifolium*.[4,5] While the structure of **2** was originally verified by synthesis, no NMR, UV, or IR data have been available for the compound.[4] Our independent spectrochemical characterization of teretifolione B (**2**) confirmed the structure, and proton-detected heteronuclear correlation experiments (HMQC and HMBC) allowed the complete assignment of all ^1H and ^{13}C NMR resonances.





The ^1H NMR spectrum of conocurvone (**1**) is very complex, with many highly overlapped resonances (Figure 1). In addition, the aromatic proton resonances (Figure 2) are flanked by satellite peaks that apparently integrated for less than one proton. The satellite peaks were originally suspected to arise from a chromatographically inseparable impurity that coeluted with conocurvone (**1**). However, the complexity and chemical shifts of

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(1) Part 11 in the series HIV Inhibitory Natural Products. For part 10, see: McKee, T. C.; Cardellina, J. H., II; Tischler, M.; Snader, K. M.; Boyd, M. R. *Tetrahedron Lett.* **1993**, *34*, 389–392.
(2) Boyd, M. R. *AIDS Etiology, Diagnosis, Treatment and Prevention*; Lippincott: Philadelphia, PA, 1988; pp 305–319.
(3) Weislow, O. S.; Kiser, R.; Fine, D. L.; Shoemaker, R. H.; Bader, J.; Boyd, M. R. *J. Natl. Cancer Inst.* **1989**, *81*, 577–586.
(4) Cannon, J. R.; Joshi, K. R.; McDonald, I. A.; Retallack, R. W.; Sierakowski, A. F.; Wong, L. C. H. *Tetrahedron Lett.* **1975**, 2795–2798.
(5) Thompson, R. H. *Naturally Occurring Quinones* III: *Recent Advances*; Chapman & Hall: London, 1987; p 208.

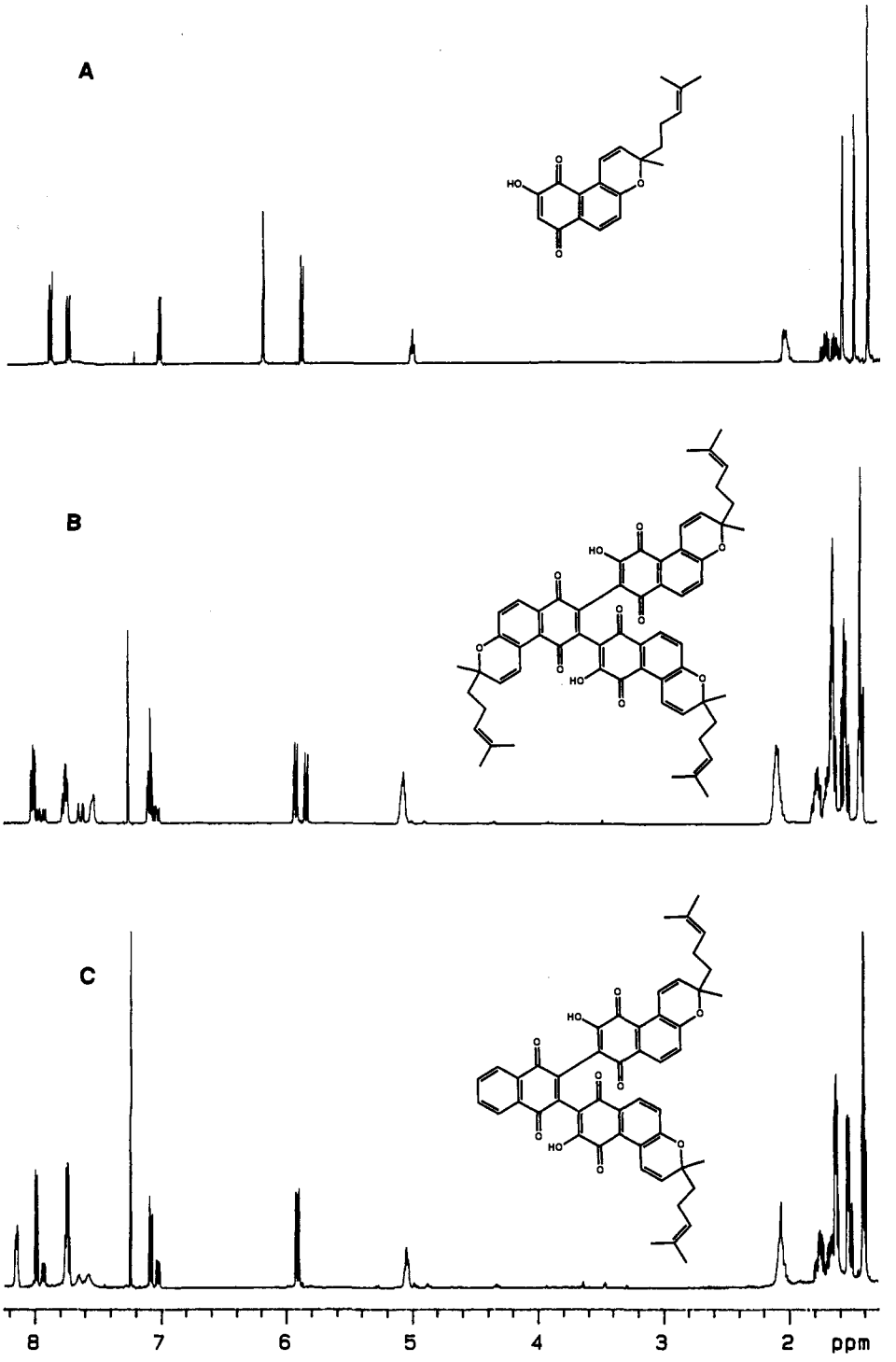


Figure 1. 500-MHz ^{1}H NMR spectra ($CDCl_3$) of (A) the monomer teretifolione B (2), (B) the naturally occurring trimer conocurvone (1), and (C) the semisynthetic derivative 4.

both the major ^{1}H NMR signals and the satellite resonances varied depending on the deuterated solvent used and the temperature of the sample. Spectra were acquired in $CDCl_3$, CD_3OD, CD_3CN, DMSO-d_6, pyridine-d_5, and benzene-d_6. A solution in benzene-d_6 provided the most complex proton NMR spectrum, while $CDCl_3$ gave the least complex spectrum. Neither heating the sample to 100 °C nor cooling to −40 °C eliminated the satellite peaks, although significant changes in the appearance and the coupling patterns of the major and minor signals were evident. In addition to changes in spectral complexity, changes in the relative integration of the major signals and satellite peaks were also observed in the different NMR solvents and at varying temperatures. These observations suggested that the conocurvone (1) sample was indeed pure and that the satellite peaks and much of the spectral complexity arose from a dynamic intramolecular process observable on the NMR time scale, such as tautomerization and/or restricted rotation about one or more bonds in the molecule.

While the ^{1}H NMR spectrum of conocurvone (1) is complex, it exhibits many similarities to the spectrum of teretifolione B (2) (Figure 1). The ^{13}C NMR spectrum of 1 also shows close correspondence to that recorded for 2, but many of the resonances occurred as overlapping clusters of peaks. The structure of conocurvone (1) is clearly related to that of teretifolione B (2), but the complex nature of the NMR data for 1 prevented a complete and straightforward structural elucidation by conven-

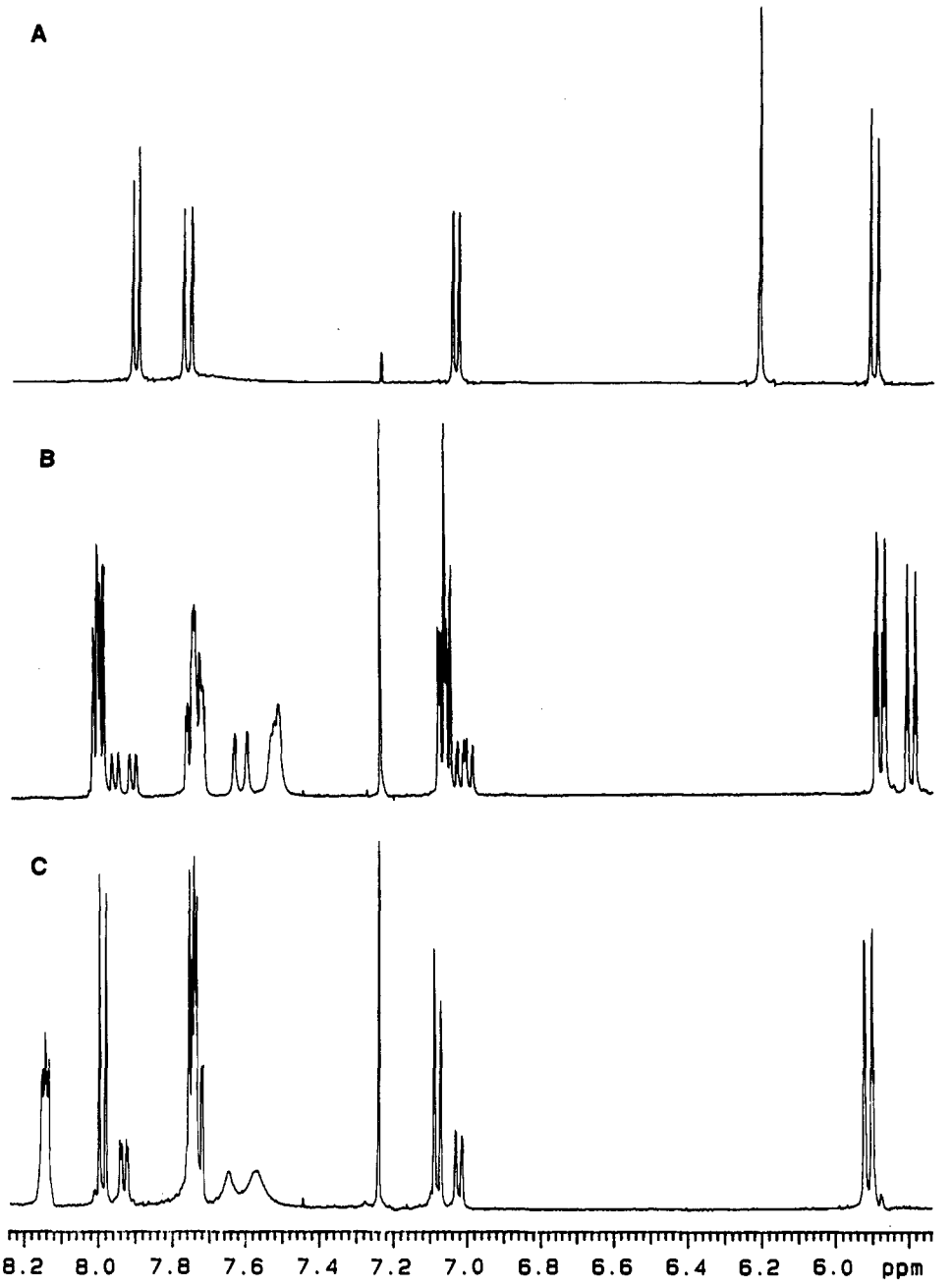


Figure 2. Expansion of the aroamtic region of the 500-MHz ^1H NMR spectra ($CDCl_3$) of (A) teretifolione B (**2**), (B) conocorvone (**1**), and (C) compound **4**.

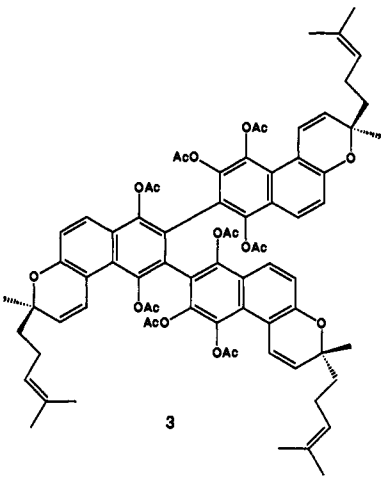
tional NMR analysis. Key information on the nature of **1** was obtained from mass spectroscopic studies. High-resolution FABMS in both the positive- and negative-ion modes revealed a unique molecular formula of $C_{60}H_{56}O_{11}$ for **1**. In the positive-ion mode, a pseudomolecular ion was observed at m/z 953.3856 for MH^+ ($C_{60}H_{57}O_{11}$), while negative-ion FABMS revealed a molecular ion at m/z 952.3872 for M^- ($C_{60}H_{56}O_{11}$) and daughter ions matching those in a B/E linked-scan at m/z 629.2535 for $C_{40}H_{37}O_7$ and m/z 323.1281 for $C_{20}H_{19}O_4$. Negative-ion FABMS, performed using a matrix of nitrobenzyl alcohol, deuterated glycerol, and deuterated methanol, produced a peak at m/z 954, which revealed that conocurvone (**1**) contained two exchangeable hydrogen atoms. A linked-scan analysis provided daughter ions at m/z 630 and 324, indicating that these fragments each contained one exchangeable hydrogen. The molecular formula, mass spectral fragmentation pattern, and NMR spectral features indicated that conocurvone (**1**) had a nonsymmetrical trimeric structure which incorporated three quinone subunits structurally related to **2**.

We surmised that conocurvone (**1**) consisted of two teretifolione B subunits joined at their C3 positions to the C2 and C3 positions of a 2-deoxyteretifolione B subunit. Structure **1** is consistent with the observed mass spectral data and would be expected to exhibit the effects of both tautomerism and restricted rotation. The two hydroxy quinone functionalities in the molecule can exist in either the ortho or para quinone forms. The relative distribution of the ortho and para quinones and their rate of interconversion could change with changes in both solvent or temperature. The planar orientation of the quinones in the two teretifolione B subunits is likely to be nearly orthogonal to the plane of the quinone in the central 2-deoxyteretifolione B portion of the molecule. Molecular models revealed that, although it is hindered, rotation about the bonds joining the two teretifolione B subunits to the central 2-deoxyteretifolione B subunit should be possible. Since each subunit in **1** is unsymmetrical and contains a chiral carbon, there are four different tautomeric forms and four different rotational atropisomers possible. In combination, this means that conocurvone (**1**) can theoretically exist in, and

interconvert among, a total of 16 different forms. The occurrence of, and NMR effects due to, similar types of keto–enol tautomerism and restricted rotation about the bonds joining subunits of other dimeric and trimeric naphthaquinone derivatives are well known.[6-8] The data presented, therefore, are fully consistent with the spectral characteristics that have been reported for these types of compounds.

Acetylation of conocurvone (1) with acetic anhydride in pyridine followed by phenyl-bonded phase HPLC of the reaction products provided a chromatogram with one main peak and many smaller peaks. The 500-MHz ^{1}H NMR spectrum of the principal peak was still complex, with many overlapping signals. Photodiode array HPLC analysis of this major peak provided a chromatogram with one major and three minor peaks and revealed that all four of the HPLC peaks had similar UV absorption profiles. While acetylation of the two hydroxyl groups in 1 would eliminate tautomeric interconversions, these results indicated that the acetate groups were not sufficiently bulky to block rotation about the bonds joining the three quinone subunits.

In an effort to eliminate the effects of tautomerism and rotatioinal interconversions, conocurvone (1) was reductively acetylated[9] with acetic anhydride, NaOAc, and Zn dust to give a mixture of peracetylated derivatives. HPLC separation of the reaction products provided octaacetate 3, with a molecular ion at m/z 1294 appropriate for $C_{76}H_{78}O_{19}$, and its ^{1}H NMR spectrum contained eight CH_3 singlets between 1.95 and 2.34 ppm due to eight acetate groups. The ^{1}H NMR signals of 3 were indicative



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of a single entity: they were sharp, less complex, and without any satellite peaks. Thus, reductive acetylation of 1 provided a product that could be chromatographically purified to a homogeneous constituent with a singlet set of NMR resonances. No sign of rotational interconversion, as suggested by the appearance of additional HPLC peaks or NMR signals, was observed with compound 3. While the peracetate derivative 3 was a single, stable material, we were unable to determine from its ^{1}H NMR characteristics which rotational orientation it possessed. The lack of protonated carbons in the region of linkage between the reduced quinone subunits in the molecule made conventional analysis by heteronuclear correlation and NOE experiments impossible.

In an effort to support the proposed structure of conocurvone (1) and to investigate the structural requirements for HIV-inhibitory activity, we undertook the semisynthesis of a structural

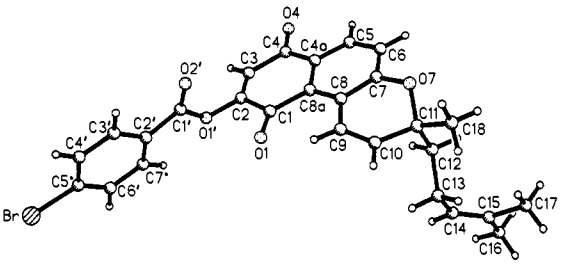
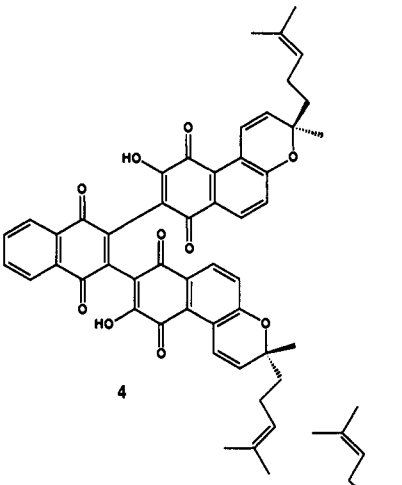


Figure 3. Computer-generated perspective drawing showing the absolute stereochemistry of the *p*-bromobenzoate derivative 6.

analog of 1. The acid-catalyzed coupling[10] of 2 equiv of teretifolione B (2) with 1,4-naphthoquinone provided a modest yield of compounds 4 and 5. It is interesting to note that the



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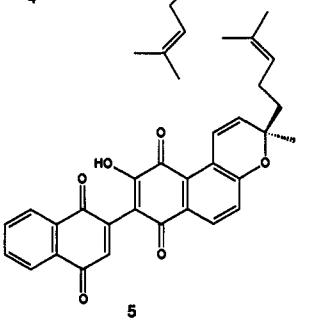

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trimer 4 had ^{1}H NMR characteristics which were very similar to those of conocurvone (1) (see Figures 1 and 2). The ^{1}H NMR resonances of 4 were complex, with many highly overlapped signals and satellite peaks. The striking similarities in the NMR spectral features of 1 and 4 suggested that the same intramolecular tautomerism and rotational isomerism that had been observed in 1 were also occurring in 4. In contrast, the synthetic dimer 5 provided sharp signals for each proton in its ^{1}H NMR spectrum.

Ultimately, the structural proof and assignment of absolute stereochemistry for conocurvone (1) was achieved through a combination of X-ray crystallographic analysis and synthetic interconversions. A computer-generated perspective drawing of 6, as determined by X-ray diffraction, is given in Figure 3. By taking advantage of the anomalous scattering power of bromine, an absolute structure refinement was performed ($\eta = -1.05[4]$), indicating an *R* configuration at C11. The molecule exhibits an extended conformation, and the pyran ring exhibits a nearly half-chair conformation with a slight disorder about the C9 and C18 atoms. The torsional angle about the (C7)C8–C9(C10) bond is about −15°, leading to an out-of-plane distance of roughly 0.5 A for C11. The plane is defined by O7 through C9 of the pyran ring.

We were then able to produce the central deoxymonomeric subunit of 1 by removal of the C2 hydroxyl group from compound 2. To achieve this critical interconversion, the *p*-bromobenzoate

(6) Waterman, P. G.; Zhong, S. M.; Jeffreys, J. A. D.; bin Zakaria, M. *J. Chem. Res. Miniprint* **1985**, 0101–0144.
(7) Jeffreys, J. A. D.; bin Zakaria, M.; Waterman, P. G.; Zhong, S. M. *Tetrahedron Lett.* **1983**, 1085–1088.
(8) Durley, R. C.; MacMillan, J.; Simpson, T. J.; Glen, A. T.; Turner, W. B. *J. Chem. Soc., Perkin Trans. I* **1975**, 163–169.
(9) Cameron, D. W.; Sidell, M. D. *Aust. J. Chem.* **1978**, *31*, 1323–1333.
(10) Jurd, L. *Aust. J. Chem.* **1980**, *33*, 1603–1610.

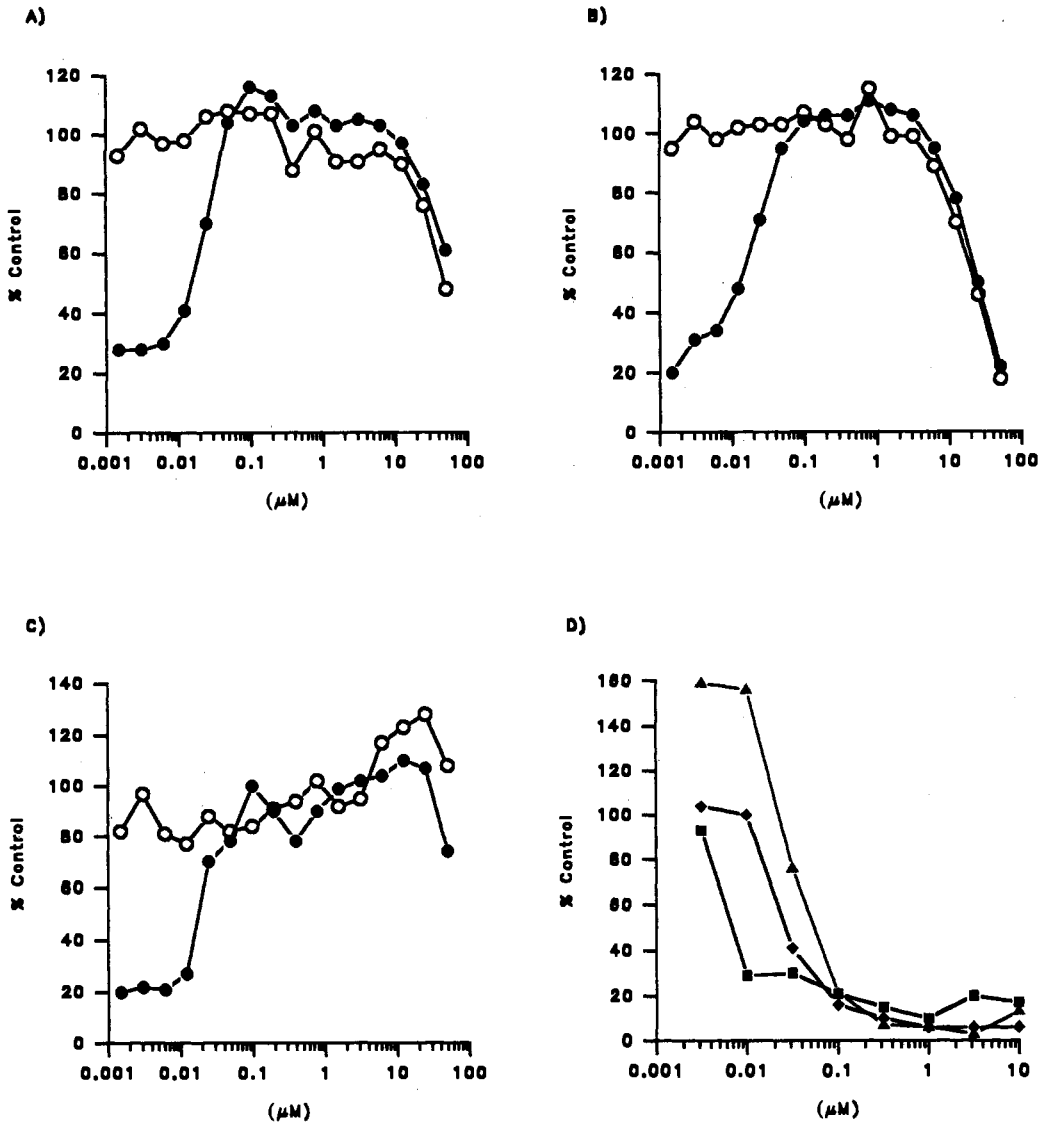

Figure 4. Graphs A, B, and C show the effects of a range of concentrations of conocurvone (**1**) upon uninfected CEM-SS cells (○) and upon CEM-SS cells infected with HIV-1 (●), as determined after 6 days in culture. Graph A depicts the relative numbers of viable CEM-SS cells, as assessed by the BCECF assay; graph B depicts the relative DNA content of the respective cultures; graph C depicts the relative numbers of viable CEM-SS cells, as assessed by the XTT assay. Graph D shows the effects of a range of concentrations of conocurvone upon indices of the infectious virus or viral replication, as determined after 4 days in culture; these indices include viral reverse transcriptase (▲), viral core protein p24 (◆), and syncytium-forming units (■). In graphs A, B, and C, the data are represented as the percent of the uninfected, nondrug treated control values. In graph D, the data are represented as the percent of the infected, nondrug treated control values.

derivative **6** was treated with thiophenol to form the bis-(thiophenol) adduct **7**. Raney nickel reduction of **7** provided the desired deoxymonomer **8**. Base-catalyzed coupling of compound **8** with 2 equiv of teretifolione B (**2**) gave compound **1**. The semisynthetically derived **1** was identical by ^{1}H NMR, IR, and UV spectra and an anti-HIV activity profile to naturally occurring conocurvone (**1**). The observation that both the natural and semisynthetic products had identical ^{1}H NMR spectra, including all of the minor satellite peaks, supported our deduction that conocurvone exists as an equilibrium mixture of tautomers and atropisomers and indicated that both compounds had the same relative configuration. The optical rotation of the semisynthetic material ($[\alpha]_D = +175°$) was in very close correspondence with that measured for the natural product ($[\alpha]_D = +184°$); therefore, we were able to assign *R* absolute stereochemistry for the three chiral centers in **1**.

Comparative testing of the natural and synthetic conocurvone (**1**) and the synthetic analog **4** in the primary screen[2,3] revealed essentially identical anti-HIV activities (EC_{50} 0.02 μM). Yet more definitive demonstration of the anti-HIV activity was obtained with a battery of interrelated assays performed as described elsewhere.[11] Cellular growth and viability in the presence and absence of various concentrations of conocurvone (**1**) were estimated[11] in uninfected and HIV-1 infected CEM-SS human lymphoblastoid cells by metabolic assays using the fluorescent probe BCECF (Figure 4A) and the XTT-tetrazolium reagent XTT (Figure 4C) and by DNA assay using the intercalating dye DAPI (Figure 4B). Concurrently, the effects of the compound upon HIV-1 replication (Figure 4D) were determined[11] by measurements of supernatant viral reverse transcriptase (RT), viral p24 antigen (p24), and syncytium-forming units (SFU). Conocurvone (**1**) completely inhibited the killing of the human CEM-SS target cells by HIV-1 (Figures 4A–C) and essentially halted HIV-1 replication (Figure 4D) in these cells. With an antiviral EC_{50} of ≤0.02 μM and a cytotoxic or growth-inhibitory IC_{50} of ≥50 μM, conocurvone yielded an extraordinary *in vitro* therapeutic index of approximately 2500 in this HIV-1/CEM-SS assay model. In sharp contrast, teretifolione B (**2**) and the synthetic dimer **5** were devoid of HIV-inhibitory activity.

(11) Gulakowski, R. J.; McMahon, J. B.; Staley, P. G.; Moran, R. A.; Boyd, M. R. *J. Virol. Methods* **1991**, *33*, 87–100.

Experimental Section

Instrumentation. NMR spectra were recorded on a Varian VXR 500 spectrometer at 25 °C unless otherwise indicated. FABMS samples were ionized with 6-kV xenon atoms and the spectra recorded with a JEOL SX102 spectrometer. A description of the other equipment and instrumentation used in these studies has been provided in previous reports.[12,13] All ^{1}H NMR J values are reported in hertz.

Collection and Extraction. The samples of *Conospermum* sp. (Spjut 7139) were collected by R. Spjut in the Gairdner mountain range of western Australia in 1981. Dried plant material (1287 g) was ground, percolated at room temperature in 1:1 CH_2Cl_2/MeOH, and then washed with 100% MeOH. Solvent was removed *in vacuo* to provide a total of 39.0 g of crude organic extract.

Chromatographic Separation. An 11.5-g portion of the organic extract was suspended in 450 mL of a 9:1 MeOH/H_2O mixture and partitioned with hexane (4 × 300 mL) to yield 2.72 g of hexane-soluble material. Addition of 45 mL of H_2O to the aqueous methanol layer and subsequent extraction with CCl_4 (4 × 300 mL) afforded 1.0 g (8.7% of the crude extract) of anti-HIV-active CCl_4 solubles. The hexane-soluble fraction was defatted by a repeat of the partitioning between hexane (800 mL) and 9:1 MeOH/H_2O (800 mL) to give an additional active methanol fraction (0.6 g, 5.2% of the crude extract).

Both active fractions were subjected to centrifugal countercurrent chromatography (Ito coil) with hexane/EtOH/EtOAc/H_2O (5:4:2:1, ascending mode). Active fractions from the countercurrent chromatograph were pooled (245 mg total) and further separated by low-pressure liquid chromatography on diol packing (YMC diol-60-I40/63) using a 2.5 × 31cm column and a gradient elution of hexane/EtOAc from 99:1 to 7:3 to give 24 mg of active material. Final purificatioin of the active component was achieved by HPLC on a phenyl-bonded phase column (Rainin Dynamax-Phenyl) with CH_3CN/H_2O (17:3, 0.1% HOAc by vol) to give 8.5 mg of conocurvone (**1**).

In a modified isolation procedure, an extract of *Conospermum* sp. was subjected to sequential application of solvent/solvent partitioning, gel permeation on Sephadex LH-20 with CH_2Cl_2/MeOH (1:1), low-pressure diol chromatography with increasingly polar mixtures of hexane/EtOAc, and C-18 HPLC using MeOH/H_2O (19:1, 0.1% HOAc by vol) to provide teretifolione B (**2**).

Conocurvone (1): $[\alpha]_D$ +184° (c 0.32, MeOH); UV λ_{max} (MeOH) 225 (log ϵ = 4.9), 275 (4.6), 289 (4.6), 405 nm (4.2); IR (film) ν_{max} 2969, 2922, 2857, 1657, 1650, 1644, 1563, 1462, 1287, 1207, 1076, 1005, 915, 866, 750 cm^{-1}; positive-ion FABMS m/z 953.3856 (MH^+, calcd for $C_{60}H_{57}O_{11}$ 953.3901), a linked-scan provided daughter ions at m/z 871.3073 (calcd for $C_{54}H_{47}O_{11}$ 871.3118) and 869.2957 (calcd for $C_{54}H_{45}O_{11}$ 869.2962); negative-ion FABMS m/z 952.3872 (M^-, calcd for $C_{60}H_{56}O_{11}$ 952.3823), a linked-scan provided daughter ions at m/z 629.2535 (calcd for $C_{40}H_{37}O_7$ 629.2539) and m/z 323.1281 (calcd for $C_{20}H_{19}O_4$ 323.1283); negative-ion FABMS using a matrix of nitrobenzyl alcohol, deuterated glycerol, and deuterated MeOH gave m/z 954 and daughter ions at m/z 630 and 324; ^{1}H NMR ($CDCl_3$) δ 1.39–1.42 (a series of overlapped CH_3 s which integrated for 9H), 1.50–1.56 (a series of overlapped CH_3 s which integrated for 9H), 1.61–1.65 (a series of overlapped CH_3 s which integrated for 9H), 1.63–1.70 (m, 3H), 1.71–1.79 (m, 3H), 2.03–2.11 (m, 6H), 5.01–5.09 (m, 3H), 5.81–5.83 (overlapped d, J = 10.5, which integrated for 1H), 5.89–5.92 (overlapped d, J = 10.5, which integrated for 2H), 6.99–7.09 (overlapped d, J = 8.5, which integrated for 3H), 7.51–7.63 (OH, exchangeable, integrated for 2H), 7.71–7.76 (overlapped d, J = 10.5, which integrated for 3H), 7.89–8.01 (overlapped d, J = 8.5, which integrated for 3H).

Teretifolione B (2): $[\alpha]_D$ +66° (c 0.1, MeOH); UV λ_{max} (MeOH) 215 (log ϵ = 4.7), 291 (4.3), 394 nm (3.8); IR (film) ν_{max} 3350 (br), 2970, 2926, 1660, 1651, 1639, 1563, 1463, 1311, 1207, 1074, 972, 844, 744 cm^{-1}; positive-ion FABMS m/z 325.1435 (MH^+, calcd for $C_{20}H_{21}O_4$ 325.1440); ^{13}C NMR (125 MHz, $CDCl_3$, number of attached H from DEPT experiment) δ 183.9 (C1, 0), 156.5 (C2, 0), 109.0 (C3, 1), 184.4 (C4, 0), 126.9 (C4a, 0), 128.6 (C5, 1), 122.1 (C6, 1), 158.1 (C7, 0), 121.5 (C8, 0), 123.3 (C8a, 0), 119.9 (C9, 1), 135.4 (C10, 1), 79.3 (C11, 0), 41.2 (C12, 2), 22.6 (C13, 2), 123.4 (C14, 1), 132.1 (C15, 0), 25.5 (C16, 3), 17.5 (C17, 3), 26.5, (C18, 3); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.40 (s, H18, Me), 1.51 (s, H17, Me), 1.60 (s, H16, Me), 1.65 (m, H12′), 1.74 (m, H12), 2.05 (m, H13, 2H), 5.03 (m, H14), 5.90 (d, H10, J = 10.5), 6.21 (s, H3), 7.04 (dd, J = 8.5, 0.7, H6), 7.48 (OH, exchangeable), 7.76 (dd, J = 10.5, 0.7, H9), 7.90 (d, J = 8.5, H5).

Acetylation of Conocurvone (1). A 5.2-mg sample of conocurvone (**1**) was stirred at room temperature for 24 h in 1 mL of pyridine and 1 mL of acetic anhydride. Ten milliliters of EtOAc was added to the reaction mixture, which was then washed successively with a 10% $CuSO_4$ solution and three times with H_2O. The EtOAc layer was dried over anhydrous Na_2SO_4 and evaporated to give 5.2 mg of product, which gave a single spot by TLC on silica plates developed with CH_2Cl_2/MeOH (19:1). HPLC on a phenyl-bonded phase column with CH_3CN/H_2O (19:1) provided a chromatogram with a series of peaks. The principal peak was collected (2.1 mg), but it gave a complex ^{1}H NMR spectrum. Reinjection of the material collected as the principal peak gave one major peak and three minor ones. The material collected as the major peak of the second HPLC separation still gave a complex ^{1}H NMR spectrum and when reanalyzed under the exact same HPLC conditions, again gave a chromatogram with one main peak and three minor ones. Photodiode array detection of the HPLC eluant showed that all four peaks had similar UV absorption profiles with a λ_{max} at 230 nm and a shoulder at 273 nm. The HPLC-purified acetylation product gave a LRMS molecular ion at m/z 1036: ^{1}H NMR ($CDCl_3$) δ 1.39–1.44 (a series of overlapped CH_3 s which integrated for 9H), 1.53–1.58 (a series of overlapped CH_3 s which integrated for 9H), 1.62–1.66 (a series of overlapped CH_3 s which integrated for 9H), 1.61–1.71 (m, 3H), 1.72–1.81 (m, 3H), 2.01–2.10 (m, 6H), 2.09–2.12 (a series of overlapped CH_3 s which integrated for 6H), 5.06 (m, 3H), 5.80–5.85 (overlapped d, J = 10.5, which integrated for 3H), 7.03–7.09 (overlapped d, J = 9.0, which integrated for 3H), 7.60–7.64 (overlapped d, J = 10.5, which integrated for 2H), 7.72–7.75 (overlapped d, J = 10.5, which integrated for 1H), 7.90–7.92 (overlapped d, J = 9.0, which integrated for 2H), 7.99–8.01 (overlapped d, J = 9.0, which integrated for 1H).

Reductive Acetylation of Conocurvone (1) To Give Compound 3. A 6.3-mg aliquot of conocurvone (**1**) in 2.5 mL of acetic anhydride with 143 mg of NaOAc and 168 mg of Zn dust was reacted at room temperature for 3.5 h. Cold H_2O was added to the reaction mixture, which was then partitioned between H_2O and CH_2Cl_2. The CH_2Cl_2 layer was dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure to give 9.5 mg of crude product. The reaction product was separated by phenyl-bonded phase HPLC with MeOH/H_2O (19:1) to give three principal peaks. The early eluting peak was further purified by HPLC on the chiral-support cyclobond β with hexane/iPrOH (19:1) to give 1.1 mg of the peracetylated product **3**: LRMS m/z 1294, appropriate for $C_{76}H_{78}O_{19}$; ^{1}H NMR ($CDCl_3$) δ 1.20 (s, 6H), 1.35 (s, 3H), 1.57 (s, 6H), 1.61 (s, 3H), 1.66 (s, 6H), 1.70 (s, 3H), 1.71–1.79 (m, 4H), 1.82–1.88 (m, 2H), 1.95 (s, 3H), 1.96 (s, 6H), 2.0–2.13 (m, 6H), 2.08 (s, 3H), 2.20 (s, 3H), 2.24 (s, 3H), 2.33 (s, 3H), 2.34 (s, 3H), 5.10 (t, J = 7.0, 2H), 5.16 (t, J = 7.0, 1H), 5.54 (d, J = 10.5, 1H), 5.55 (d, J = 10.2, 1H), 5.65 (d, J = 10.2, 1H), 6.54 (d, J = 9.3, 2H), 7.12–7.22 (m, 6H), 7.62 (d, J = 9.3, 1H).

Coupling of Teretifolione B (2) and 1,4-Naphthaquinone To Provide Compounds 4 and 5. A 61-mg aliquot of teretifolione B (**2**), 16 mg of 1,4-naphthoquinone, and 1 mL of glacial HOAc were stirred for 15 h in a refluxing water bath. The mixture was evaporated to dryness under reduced pressure and chromatographed on Sephadex LH-20 with CH_2Cl_2/MeOH (1:1). Early eluting fractions which contained a new, more polar spot by TLC (SiO_2 plates developed with CH_2Cl_2/MeOH, 9:1, R_f = 0.13) were pooled and purified by HPLC on a phenyl-bonded phase column with CH_3CN/H_2O (17:3, 0.1% HOAc by vol) to give 7 mg of the trimer **4**. Later eluting fractions from the Sephadex LH-20 column were chromatographed on diol-bonded phase packing to give 9 mg of compound **5**. Compound **4** had the following spectral features: FAB-HRMS m/z 803.2834 (MH^+, calcd for $C_{50}H_{43}O_{10}$ 803.2856); ^{1}H NMR ($CDCl_3$) δ 1.40–1.43 (a series of CH_3 s which integrated for 6H), 1.51–1.55 (a series of CH_3 s which integrated for 6H), 1.62–1.64 (a series of CH_3 s which integrated for 6H), 1.64–1.71 (m, 2H), 1.73–1.81 (m, 2H), 2.04–2.11 (m, 4H), 5.03–5.08 (m, 2H), 5.91–5.93 (overlapped d, J = 10.5, which integrated for 2H), 7.02–7.10 (overlapped d, J = 8.5, which integrated for 2H), 7.57 (OH, exchangeable), 7.66 (OH, exchangeable), 7.73–7.76 (m, 4H), 7.93–8.01 (overlapped d, J = 8.5, 2H), 8.14–8.16 (m, 2H).

Compound **5** had the following spectral features: FABHRMS m/z 481.1635 (MH^+, calcd for $C_{30}H_{25}O_6$ 481.1651); ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 3H), 1.55 (s, 3H), 1.64 (s, 3H), 1.65–1.72 (m, 1H), 1.76–1.83 (m, 1H), 2.07–2.13 (m, 2H), 5.08 (t, J = 7.0, 1H), 5.97 (d, J = 10.5,

(12) Gustafson, K. R.; Cardellina, J. H., II; McMahon, J. B.; Gulakowski, R. J.; Ishitoya, J.; Szallasi, Z.; Lewin, N. E.; Blumberg, P. M.; Weislow, O. S.; Buetler, J. A.; Buckheit, R. W., Jr.; Cragg, G. M.; Cox, P. A.; Bader, J. P.; Boyd, M. R. *J. Med. Chem.* **1992**, *35*, 1978–1986.

(13) Gustafson, K. R.; Cardellina, J. H., II; McMahon, J. B.; Pannell, L. K.; Cragg, G. M.; Boyd, M. R. *J. Org. Chem.* **1992**, *57*, 2809–2811.

1H), 7.03 (s, 1H), 7.11 (d, J = 8.5, 1H), 7.74 (m, 2H), 7.82 (d, J = 10.5, 1H), 8.00 (d, J = 8.5, 1H), 8.11 (m, 2H).

Preparation of the *p*-Bromobenzoate Derivative 6. In a typical reaction, 20.7 mg of teretifolione B (**2**) was stirred in 3 mL of CH_2Cl_2 with 62.1 mg of *p*-bromobenzoyl chloride and 1.0 mL of Et_3N. The reaction was stopped after 15 min, and the mixture was washed with 6 × 1 mL of H_2O. The organic layer was evaporated to dryness and triturated with an EtOAc/hexane mixture and the insoluble aromatic acid filtered off. The filtrate was adsorbed onto C-18 packing and then eluted with increasing concentrations of MeOH in H_2O. The *p*-bromobenzoate derivative **6** (24.9 mg) eluted with 95% MeOH. Compound **6** crystallized from an acetone/H_2O mixture: HREIMS m/z 506.0702 (calcd for $C_{27}H_{23}BrO_5$ 506.0729); ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 3H), 1.55 (s, 3H), 1.65 (s, 3H), 1.70 (m, 1H), 1.77 (m, 1H), 2.09 (m, 2H), 5.07 (br t, J = 7.0, 1H), 5.88 (d, J = 10.3, 1H), 6.79 (s, 1H), 7.01 (d, J = 8.2, 1H), 7.68 (m, 2H), 7.75 (d, J = 10.3, 1H), 7.98 (d, J = 8.2, 1H), 8.03 (m, 2H).

Preparation of the Bis(thiophenol) Derivative 7. Into 50 mL of EtOAc were added 108 mg of compound **6**, 6 mL of Et_3N, and 28 drops of thiophenol. The reaction mixture was stirred at room temperature for 1 h and then evaporated to dryness. Step-gradient vacuum liquid chromatography[14] (VLC) on C-18 packing provided 93.4 mg of product in the 100% MeOH fraction. Final purification by HPLC on a phenyl column with MeOH/H_2O (19:1) yielded 58.4 mg of compound **7**: HREIMS m/z 524.1486 (calcd for $C_{32}H_{28}S_2O_3$ 524.1480); IR (film) ν_{max} 2917, 2849, 1663, 1583, 1564, 1466, 1440, 1283, 1135 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.31 (s, 3H), 1.52 (s, 3H), 1.62 (s, 3H), 1.63 (m, 1H), 1.73 (m, 1H), 2.04 (m, 2H), 5.03 (br t, J = 7.0, 1H), 5.71 (d, J = 10.5, 1H), 6.95 (d, J = 8.5, 1H), 7.04 (d, J = 10.5, 1H), 7.22–7.31 (m, 6H), 7.35–7.37 (m, 4H), 7.78 (d, J = 8.5, 1H).

Raney Nickel Reduction of 7 To Generate Compound 8. Fresh Raney nickel was prepared by washing and sonicating three times with excess MeOH. A suspension of 480 mg of washed Raney nickel in 5 mL of MeOH was brought to reflux. A solution of 5.2 mg of bis(thiophenol) **7** in MeOH was slowly added to the refluxing Raney nickel solution. Best results were obtained by waiting for the disappearance of the red coloration before adding more of the solution containing **7**. The reaction mixture was refluxed for a total of 1 h, filtered, and evaporated to dryness. Purification by step-gradient VLC on C-18 gave 22.6 mg of compound **8** in the MeOH/H_2O (19:1) fraction: HREIMS m/z 308.1401 (calcd for $C_{20}H_{20}O_3$ 308.1412); UV λ_{max} (MeOH) 225 (log ϵ = 4.4), 298 (3.7), 429 nm (3.5); IR (film) ν_{max} 2965, 2923, 2853, 1655, 1611, 1576, 1562, 1458, 1437, 1300, 1192 cm^{-1}; ^{13}C NMR ($CDCl_3$, assignments made from HMQC, HMBC, and DEPT experiments) δ 188.0 (C1), 140.0 (C2*), 137.3 (C3*), 184.3 (C4), 126.2 (C4a), 128.8 (C5), 121.2 (C6), 159.2 (C7), 120.7 (C8), 129.3 (C8a), 120.1 (C9), 134.2 (C10), 79.5 (C11), 41.3 (C12), 22.6 (C13), 123.5 (C14), 132.2 (C15), 25.6 (C16), 17.6 (C17), 26.7 (C18) (the asterisk indicates that the assignments for C2 and C3 may be reversed); ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 3H), 1.53 (s, 3H), 1.63 (s, 3H), 1.63 (m, 1H), 1.73 (m, 1H), 2.08 (m, 2H), 5.05 (br t, J = 7.0, 1H), 5.88 (d, J = 10.5, 1H), 6.80 (d, J = 10.0, 1H), 6.83 (d, J = 10.0, 1H), 7.05 (d, J = 8.5, 1H), 7.79 (d, J = 10.5, 1H), 7.92 (d, J = 8.5, 1H).

Coupling of Teretifolione B (2) with Compound 8 To Generate Conocurvone (1). In a typical reaction, 5 mg of compound **8** was mixed with 10 mg of teretifolione **B** (**2**) in 0.9 mL of pyridine and the reaction solution heated at *ca.* 80 °C for 90 min. The reaction mixture was then evaporated to dryness and purified by HPLC on a phenyl-bonded phase column, eluting with MeCN/H_2O (17:3, 0.05% TFA by vol). Final HPLC purification on the same column using MeCN/H_2O (9:1, 0.05% TFA by vol) provided 1.2 mg of material ($[\alpha]_D$ = **175°**, *c* 0.09, MeOH) that by IR, UV, ^{1}H NMR, HPLC retention, and an anti-HIV activity profile was identical to naturally occurring conocurvone (**1**).

Single-Crystal X-ray Diffraction Analysis of Teretifolione B *p*-Bromobenzoate (6). A colorless crystal measuring 0.10 × 0.20 × 0.45 mm was grown from an acetone/water mixture and was used to collect a low-temperature (–40 °C) X-ray crystallographic data set using Mo Kα radiation. Preliminary axial photos revealed orthorhombic symmetry, and least-squares analysis of diffractometer-measured 2θ values gave a = 6.112(2), b = 15.534(10), and c = 24.468(14) Å. Systematic extinctions uniquely indicated $P2_12_12_1$, and the density indicated that one molecule of composition $C_{27}H_{23}BrO_5$ formed the asymmetric unit. A total of 1924 unique reflections (reflections and Friedel pairs) were collected using ω scans with a Siemens R3M diffractometer. Of these, 1183 (61%) were judged observed ($|F_o| \geq 4\sigma(F_o)$) and used in subsequent calculations. A full-matrix least-squares refinement gave a final R_f = 4.88% and a w_R = 4.34%, where $w = 1/(\sigma^2F) + 0.0002(F)^2$). Hydrogens were placed at ideal positions and refined using rigid body techniques. Lorentz and polarization corrections, but no absorption corrections, were applied to the data. Additional crystallographic details are available and described in the supplementary material.

Anti-HIV Evaluations. DMSO solutions of the pure compounds and chromatographic fractions were tested in the XTT-based *in vitro* anti-HIV assay, experimental details of which have been reported previously.[2,3] The confirmatory assays were also performed as described elsewhere.[11]

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Supplementary Material Available: ^{13}C NMR spectra for **1** and **2**, ^{1}H NMR of semisynthetic **1**, and FABMS of natural and semisynthetic **1** as well as tables of atomic coordinates, thermal parameters, interatomic distances, angles, and torsional angles for **6** (13 pages). Ordering information is given on any current masthead page.

(14) Coll, J. C.; Bowden, B. F. *J. Nat. Prod.* **1986**, *49*, 934–936.