JOURNAL OF
MEDICINAL
CHEMISTRY

Volume 36, Number 26 December 24, 1993

Expedited Articles

The Inophyllums, Novel Inhibitors of HIV-1 Reverse Transcriptase Isolated from the Malaysian Tree, *Calophyllum inophyllum* Linn

Ashok D. Patil,* Alan J. Freyer, Drake S. Eggleston, R. Curtis Haltiwanger, Mark F. Bean, Paul B. Taylor, Mary Jo Caranfa, Ann L. Breen, Henry R. Bartus, Randall K. Johnson, Robert P. Hertzberg, and John W. Westley

Departments of Biomolecular Discovery, Analytical Sciences, Physical and Structural Chemistry, Molecular Virology, and Host Defense, SmithKline Beecham Pharmaceuticals R & D, King of Prussia, Pennsylvania 19406

Received July 9, 1993•

As part of a search for novel inhibitors of HIV-1 reverse transcriptase, the acetone extract of the giant African snail, *Achatina fulica*, was shown to be active. Fractionation of the extract yielded inophyllums A, B, C, and E and calophyllolide (**1a**, **2a**, **3a**, **3b**, and **6**), previously isolated from *Calophyllum inophyllum* Linn., a known source of nutrition for *A. fulica*. From a methanol/methylene chloride extract of *C. inophyllum*, the same natural products in considerably greater yield were isolated in addition to a novel enantiomer of soulattrolide (**4**), inophyllum P (**2b**), and two other novel compounds, inophyllums G-1 (**7**) and G-2 (**8**). The absolute stereochemistry of inophyllum A (**1a**) was determined to be 10(*R*), 11(*S*), 12(*S*) from a single-crystal X-ray analysis of its 4-bromobenzoate derivative, and the relative stereochemistries of the other inophyllums isolated from *C. inophyllum* were established by a comparison of their ^{1}H NMR NOE values and coupling constants to those of inophyllum A (**1a**). Inophyllums B and P (**2a** and **2b**) inhibited HIV reverse transcriptase with IC_{50} values of 38 and 130 nM, respectively, and both were active against HIV-1 in cell culture (IC_{50} of 1.4 and 1.6 μM). Closely related inophyllums A, C, D, and E, including calophyllic acids, were significantly less active or totally inactive, indicating certain structural requirements in the chromanol ring. Altogether, 11 compounds of the inophyllum class were isolated from *C. inophyllum* and are described together with the SAR of these novel anti-HIV compounds.

Introduction

Human immunodeficiency virus (HIV) is the cause of acquired immune deficiency syndrome (AIDS), and the development of anti-HIV agents is considered to be one of the most important approaches toward effective therapy for AIDS. HIV reverse transcriptase (HIV RT), an enzyme involved in replication of the HIV genome, is the target of clinically useful anti-HIV compounds such as AZT and ddI.[1,2] AZT and related nucleoside analogs have limited utility against AIDS and produce serious side effects such as anemia and leukopenia. In addition, the development of resistant strains of HIV-1 RT to these inhibitors has been reported recently.[3,4] We initiated a screen using recombinant purified HIV RT to look for novel non-nucleoside inhibitors from various sources. During the course of screening various natural product extracts for HIV RT inhibition, we tested extracts of the giant African snail, *Achatina fulica*, collected in the Seychelles.

Chemistry

The wet snails were thawed and removed from their shells and extracted sequentially with acetone and water. Bioassay-guided fractionation of the acetone extract that showed HIV RT activity (IC_{50} = 6 μg/mL) over a column of silica gel followed by extensive preparative TLC and HPLC led to the isolation of a series of coumarin derivatives with potent inhibitory activity. On silica gel plates, these compounds charred upon treatment with vanillin/H_2SO_4 to give characteristic blue spots which intensified in color over 12 h to a deep ink-blue appearance.

• Abstract published in *Advance ACS Abstracts*, December 1, 1993.

0022-2623/93/1836-4131$04.00/0

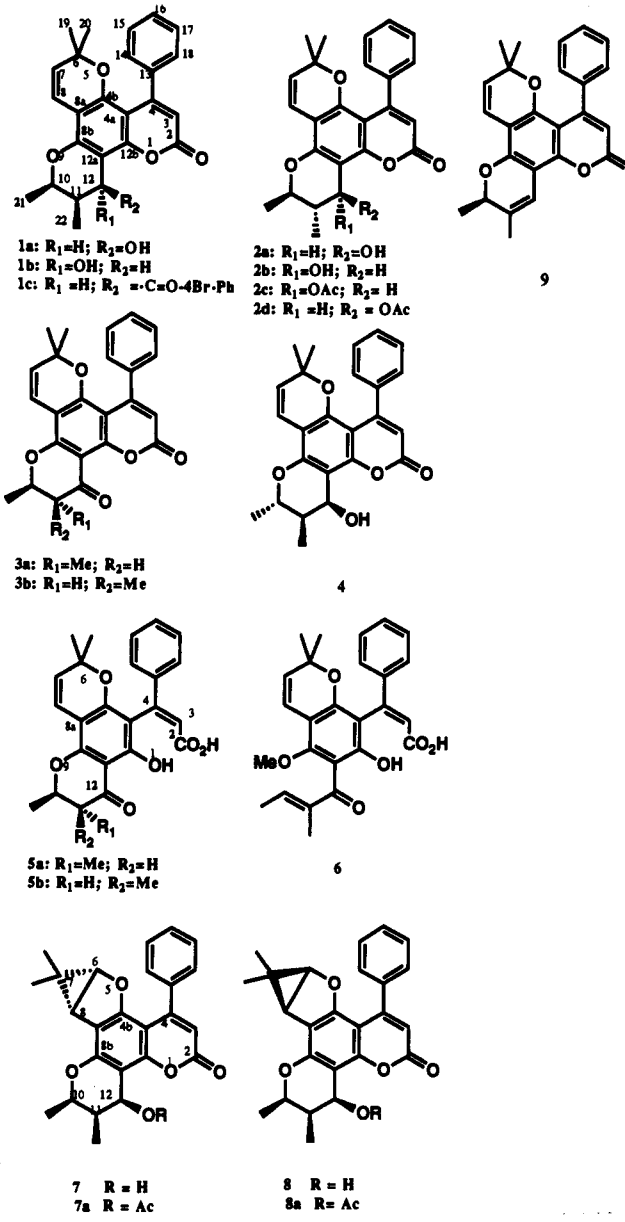

Figure 1. Chemical constituents of *C. inophyllum*.

The compounds were identified as inophyllums A (**1a**), D (**1b**), B (**2a**), C (**3a**), and E (**3b**) and calophyllolide (**6**) (Figure 1). They belong to a known class of coumarins, previously isolated from the leaves of *Calophyllum inophyllum* Linn.[5-7] Antibacterial, antiinflammatory, and phagocytosis stimulant activities were reported for this plant[8] which traditionally has been used for the treatment of eye diseases and as an ingredient in aromatic powders and liniments. Previous phytochemical studies of the genus *Calophyllum* had revealed it to be a rich source of secondary metabolites. Xanthones, steroids, triterpenes, coumarins, and benzopyrans were among compounds reported earlier from *Calophyllum* species, although no antiviral activity had previously been associated with this genus until the recent report by Boyd and co-workers on the calanolides.[9,10] Since the giant African snail is known to feed on leaves on the *Calophyllum* plant,[11] we reasoned that the natural products which we had isolated initially may have originated from material consumed by the snail. Since the active compounds inophyllums A and B (**1a** and **2a**; IC_{50} of 30 μM and 38 nM, respectively) were present in small amounts in the *A. fulica* extract and there was not sufficient material to perform all desired assays, arrangements were made to collect leaves of *C. inophyllum* in Malaysia.

The dried leaves and twigs of *C. inophyllum* were extracted with $MeOH/CH_2Cl_2$ (1:1). The organic extract, which demonstrated activity against HIV RT, was triturated with methylene chloride. Bioassay-guided fractionation of the enriched HIV RT active plant extract using silica gel and reversed-phase C-18 silica gel open-column chromatography followed by silica gel PTLC and HPLC led to the isolation and characterization of the six compounds isolated from *A. fulica* plus three novel compounds: the 12-epimer of inophyllum B, to which we gave the name inophyllum P (**2b**), and inophyllums G-1 (**7**) and G-2 (**8**). Calophyllic acid (**5a**) and isocalophyllic acid (**5b**) were also isolated, and their stereochemistries were determined by X-ray crystallography.[12]

Structure Determination

The structures of calophyllolide and inophyllums A–E were identified by means of a variety of spectroscopic techniques and were then verified by direct comparison of spectral data with literature values.[5-7] The absolute stereochemistry of the inophyllums is of interest, particularly that of inophyllums A, B, and P (**1a**, **2a**, and **2b**), because of their activity in the HIV RT assay.

Inophyllum P (2b). This compound was isolated as an optically active white solid, $[\alpha]_D$ +31.4°. The HRCIMS (methane) displayed a $(M + H)^+$ at m/z 405.1732 indicating a molecular formula of $C_{25}H_{24}O_5$. The LRCIMS had only one significant peak at m/z 386 ($M - H_2O$). The UV spectrum (λ_{max} 235, 280, 286, and 337 nm) is quite similar to that of inophyllums A, B, and D (**1a**, **2a**, and **1b**). The IR spectrum shows bands which are ascribed to a hydroxyl group (3435 cm^{-1}), an α,β-unsaturated lactone (1719 cm^{-1}), and a monosubstituted benzene ring (765 and 703 cm^{-1}).

The ^1H NMR spectrum of inophyllum P (**2b**) is very similar to that of inophyllum B (**2a**) with chemical shift and coupling constant discrepancies appearing solely among the chromanol ring protons (Table I). Compared to **2a**, the H-10 and H-12 protons of **2b** appear 0.3 ppm further downfield while H-11 is 0.2 ppm further upfield. A COSY NMR spectrum of inophyllum P (**2b**) shows correlations between the multiplet at δ 1.79 (H-11) and H-10 at δ 4.29, H-12 at δ 5.04, and CH_3-22 at δ 1.17.

The ^{13}C NMR spectrum of **2b** is consistent with a lactone carbonyl at δ 160.1, 16 sp^2 carbons between δ 156.4 and 103.6, three oxygenated carbons near δ 70, and five upfield aliphatic carbons (four of which are methyls). This spectrum is nearly superimposable with the ^{13}C spectrum of inophyllum B (**2a**) with the exception of the C-10, C-11, and C-12 chemical shifts which are all located in the chromanol ring (Table II).

Inophyllum P (**2b**) was readily acetylated with acetic anhydride/pyridine to give the monoacetate **2c**, 42-Da more massive by CIMS, the ^1H NMR spectrum of which displays a three-proton singlet at δ 2.12 and a downfield-shifted H-12. Thus, inophyllum P (**2b**) must be a dihydro derivative of the C-12 ketone inophyllum C (**3a**), as is inophyllum B (**2a**). Comparison of the ^1H NMR shifts of the chromanol ring protons (Figure 2) of inophyllums A (**1a**), D (**1b**), and E (**3b**) suggests that D and A are hydroxyl analogs of inophyllum E (**3b**).

Stereochemistry. The absolute configuration of inophyllum A (**1a**) was established by X-ray analysis of inophyllum A 4-bromobenzoate **1c** which was prepared

Table I. 400-MHz ^{1}H NMR Chemical Shifts[a] for Compounds **2a**, **2b**, **5a**,[b] **5b**,[b] **7a**, and **7b** in $CDCl_3$

proton	2a	2b	5a	5b	7	8
3	5.97, s	5.97, s	6.40, s	6.41, s	6.01, s	6.02, s
Ph-4	7.41, 7.31, m	7.42, 7.30, m	7.35, 7.28, m	7.36, 7.26, m	7.41, 7.31, m	7.42, 7.32, m
6					4.23, d, J = 5.7 Hz	4.21, d, J = 5.7 Hz
7	5.37, d, J = 10.0 Hz	5.37, d, J = 10.0 Hz	5.4, d, J = 10.0 Hz	5.39, d, J = 10.0 Hz		
8	6.53, d, J = 10.0 Hz	6.54, d, J = 10.0 Hz	6.50, d, J = 10.0 Hz	6.51, d, J = 10.0 Hz	2.46, d, J = 5.7 Hz	2.42, d, J = 5.7 Hz
10	3.96, dq, J = 6.4, 9.1 Hz	4.29, dq, J = 6.3, 10.6 Hz	4.21, dq, J = 6.4, 11.4 Hz	4.58, dq, J = 6.3, 3.2 Hz	4.46, ddq, J = 0.8, 3.8, 6.8 Hz	4.40, ddq, J = 0.8, 3.3, 6.8 Hz
11	1.97, ddq, J = 6.8, 9.1, 7.8 Hz	1.79, ddq, J = 3.3, 7.0, 10.6 Hz	2.58, dq, J = 6.9, 11.3 Hz	2.57, dq, J = 7.3, 3.2 Hz	*2.34*, ddq, J = 3.8, 4.8, 7.1 Hz	*2.33*, ddq, J = 3.3, 5.3, 7.1 Hz
12	4.79, d, J = 7.8 Hz	5.04, d, J = 3.3 Hz			5.12, dd, J = 4.8, 0.8 Hz	5.18, dd, J = 5.3, 0.8 Hz
19	0.97, s	0.93, s	0.92, s	0.93, s	1.05, s	1.05, s
20	0.91, s	0.93, s	1.24, s	1.24, s	0.71, s	0.73, s
21	1.47, d, J = 6.4 Hz	1.44, d, J = 6.3 Hz	1.50, d, J = 6.2 Hz	1.39, d, J = 6.6 Hz	1.45, d, J = 6.8 Hz	1.45, d, J = 6.8 Hz
22	1.18, d, J = 6.8 Hz	1.17, d, J = 7.0 Hz	1.18, d, J = 6.9 Hz	1.17, d, J = 7.4 Hz	1.20, d, J = 7.1 Hz	1.18, d, J = 7.1 Hz

[a] Assigned by COSY, HMQC, HMBC, and NOE experiments. [b] Spectra obtained in $MeOD_4/CDCl_3$ (1:1) and chemical shifts reported as averaged rotamers.

Table II. 100-MHz ^{13}C NMR Data[a] for Inophyllums **1a**, **1b**, **2a**, **2b**, **3a**, **3b**, **5a**, **5b**, **7**, and **8** and Calophyllolide (**6**)

carbon	1a	1b	2a	2b	3a	3b	5a[a]	5b	6	7	8
2	160.3	160.7	160.6	160.1	159.6	159.4	168.3	168.3	159.4	160.5	160.5
3	111.4	111.7	111.7	111.5	113.5	113.3	121.1	121.0	114.4	111.2	111.2
4	156.2	156.4	156.3	156.4	159.4	159.3	148.5	148.6	155.0	155.0	154.9
4a	103.1	103.3	103.1	103.6	103.4	102.7	108.6	108.5	105.6	99.0	98.9
4b	150.9	151.0	151.1	150.9	155.6	155.6	159.1	159.2	151.9	158.4	158.4
6	76.8	77.1	76.7	77.2	78.6	78.6	78.6	78.6	77.4	72.9	72.8
7	127.1	127.1	127.1	127.6	127.2	127.4	126.6	126.5	129.0	13.0	13.0
8	115.9	115.9	116.0	115.9	115.2	115.2	115.8	115.8	116.0	29.4	29.3
8a	106.3	106.0	106.0	106.3	105.4	105.4	101.7	101.1	110.7	112.0	111.9
8b	152.5	153.9	153.7	153.7	155.1	155.3	160.4	160.8	156.0	153.4	153.8
10	75.7	71.1	73.0	76.9	79.7	77.2	79.5	76.7	143.7	75.9	75.7
11	35.5	37.2	38.2	40.4	47.3	45.9	46.0	44.7	140.1	35.6	35.8
12	62.6	64.5	61.8	67.1	190.0	191.5	199.4	201.5	194.2	62.7	63.4
12a	105.5	103.9	106.1	106.1	103.9	103.8	101.9	101.8	115.2	106.1	106.0
12b	154.0	154.6	153.6	154.1	154.9	154.8	156.9	156.7	152.0	153.2	153.2
13	139.9	139.9	140.0	139.9	139.9	139.8	141.1	141.0	139.7	137.6	137.6
14	127.2	127.3	127.3	127.3	127.5	127.6	127.4	127.4	127.4	127.7	127.7
15	127.2	127.3	127.3	127.3	127.5	127.6	128.7	128.6	127.5	127.7	127.7
16	127.5	127.5	127.6	127.4	127.7	127.3	129.3	129.2	127.7	128.8	128.8
17	127.2	127.3	127.3	127.3	127.5	127.6	128.7	128.6	127.5	127.7	127.7
18	127.2	127.3	127.3	127.3	127.5	127.6	127.4	127.4	127.4	127.7	127.7
19	26.7	26.9	26.9	26.5	27.2	27.3	28.0	28.2	27.0	22.5	22.6
20	26.7	26.8	26.8	26.9	27.4	27.2	28.0	28.2	27.0	12.7	12.6
21	16.0	17.5	18.8	18.9	19.6	16.0	19.8	16.6	15.1	15.9	16.0
22	9.7	9.1	12.5	15.0	10.6	9.1	10.4	9.6	10.7	10.5	9.6
									62.5[a]		

[a] Assignments based on the HMQC, HMBC, and GASPE experiments. [b] Spectra recorded in $CD_3OD/CDCl_3$ (1:1) and chemical shifts reported as averaged rotamers. [c] Methoxy resonance.

by treatment of **1a** with 4-bromobenzoyl chloride in the presence of 4-(*N*,*N*-dimethylamino)pyridine. Once an absolute configuration of inophyllum A (**1a**) was established, we determined the stereochemistry of the chromanol ring in all other inophyllums by comparing their ^{1}H NMR NOEs and coupling constants with those of inophyllum A (**1a**). A systematic study of the coupling constants and NOEs observed with these compounds leads to some stereochemical generalizations. The coupling constants and NOEs (as a percentage of the maximum possible NOE) are presented in Figure 2 (only the chromanol and chromanone rings are depicted).

The proton NMR assignments of inophyllums A–E and P agree well with literature values.[5-7] Inophyllum P (**2b**) was originally thought to be soulattrolide, based on their identical NMR spectra, until it was determined that inophyllum P possesses an optical rotation opposite (and nearly equal) in sign to that published for soulattrolide (**4**).[7] Therefore, inophyllum P (**2b**) is the enantiomer of soulattrolide and the epimer of inophyllum B (**2a**). This was confirmed by the reduction of inophyllum C (**3a**) with an excess amoung of $NaBH_4$ in MeOH at room temperature. Subsequent workup yielded inophyllum B (**2a**) and the C-12 epimer inophyllum P (**2b**), whose ^{1}H and ^{13}C NMR spectra are identical with those of the natural product.

Inophyllum C (**3a**) and its reduction products, alcohols B and P, exhibit a large coupling constant (9–11 Hz) and a relatively small percentage of NOE (2–7%) between vicinal protons H-10 and H-11, indicating a trans diaxial relationship between them. Inophyllum E (**3b**) and its reduction products, alcohols **1a** and **1b**, possess small vicinal coupling constants (2–4 Hz) and large NOEs (10–25%) between protons H-10 and H-11, indicating an axial–equatorial relationship between them. The α alcohols, inophyllums D and P (**1b** and **2b**), exhibit small coupling constants between protons H-11 and H-12 (2–4 Hz). The β alcohols, inophyllums A and B (**1a** and **2a**), possess larger H-11/H-12 coupling constants (5–8 Hz).

For all of the inophyllums, there are small NOEs observed between diaxial groups, suggesting that the chromanol ring is undergoing constant motion. This complicated the assignment of relative stereochemistry significantly. Attempts to slow this motion by lowering

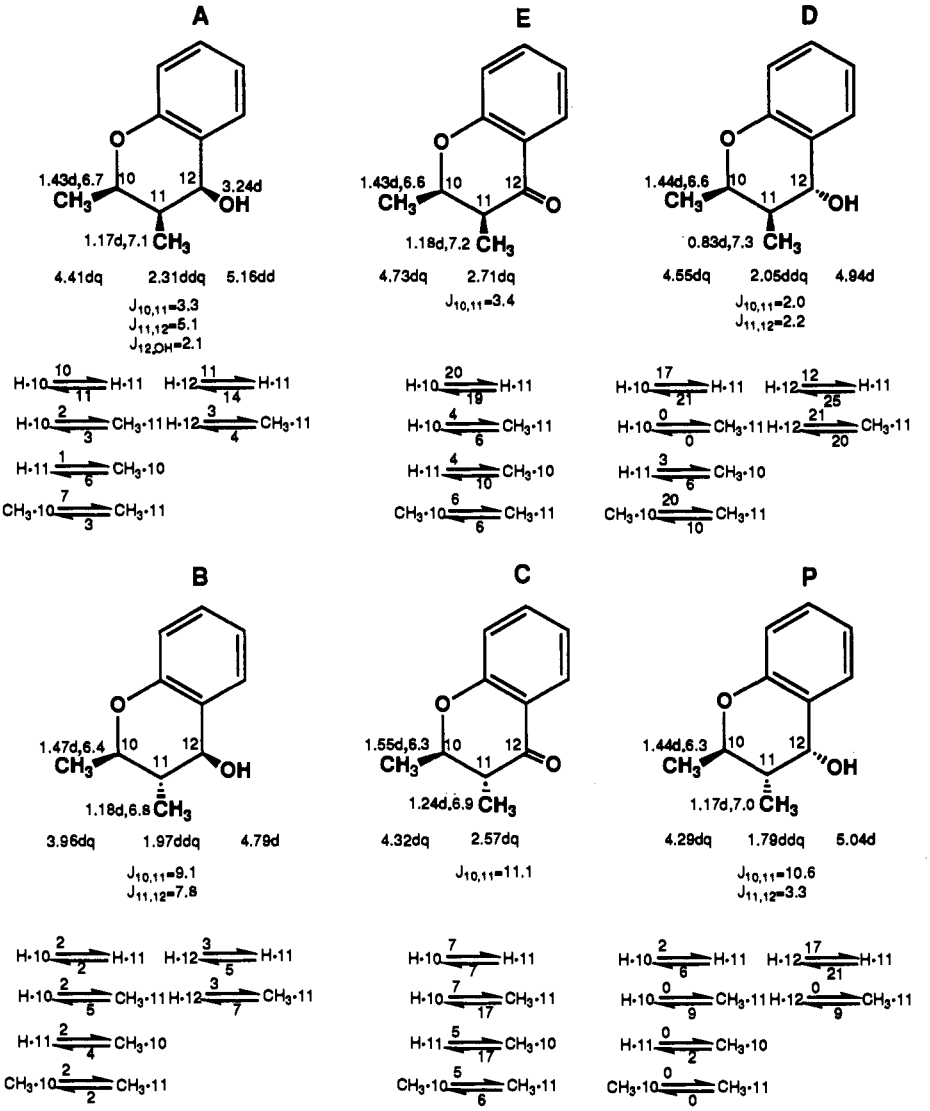

Figure 2. Inophyllum stereochemistry; a summary of the chromanol ring chemical shifts, coupling constants, and quantitative NOE results for inophyllums A, B, C, D, E, and P. Chemical shifts were secondarily referenced to TMS via the chloroform solvent signal and are presented in units of ppm on the δ scale. NOE results are shown as a percentage of the maximum possible effect with the arrows pointing from the saturated proton to the enhanced proton.

the temperature of the solution to −62 °C in deuterated chloroform were unsuccessful. In order to go to lower temperatures, inophyllum P (**2b**) was dissolved in deuterated methylene chloride. Dehydration occurred instantly resulting in the formation of 11,12-anhydroinophyllum P (**9**), whose ^{1}H NMR spectrum reveals the disappearance of the H-11 resonance and the downfield displacements of the C-22 methyl group (δ 1.89) and H-12 (δ 6.64).[6] Since all NMR solvents were treated with basic alumina, the formation of **9** from **2b** is unlikely to be due to the presence of DCl.

Calophyllic Acid (5a) and Isocalophyllic Acid (5b). The structures of these acids were determined by X-ray crystallography. To our knowledge, ^{1}H and ^{13}C NMR data for these compounds have not been reported. Both the proton and carbon NMR spectra of **5a** and **5b** (in CD_3OD/$CDCl_3$ at room temperature) show two sets of peaks of nearly equal intensity and virtually identical chemical shifts due to the presence of two rotational isomers in solution for each compound resulting from hindered rotation abut the bond connecting the acrylic acid (C-4/5). When ^{1}H and ^{13}C NMR spectra were recorded at 60 °C, the two sets of resonances coalesced. This observation may be explained by the presence of two slowly interconverting rotamers and is consistent with the observation of two independent, conformationally distinct molecules seen in the X-ray crystallographic study.[12]

Inophyllums G-1 (7) and G-2 (8). From a TLC homogeneous fraction, compounds **7** and **8** cocrystallized as fine needles. Examination of their ^{1}H NMR spectra indicated that they were clearly a mixture of two very similar compounds. The mixture was resolved by means of silica gel HPLC which yielded pure inophyllums G-1 and G-2 (**7** and **8**). Both compounds were isolated as optically active solids with specific rotations of 174.20° and −49.2°, respectively. IR spectra of compounds **7** and **8** are similar to that of inophyllum A (**1a**) and show bands at 3440 (OH), 1717 (α,β-unsaturated lactone), 770, and 703 cm^{-1} (monosubstituted benzene ring). The LRCIMS (methane) spectra indicated an $(M + H)^+$ ion at m/z 405; deuterium exchange (CI, ND_3) revealed one exchangeable hydrogen for both compounds **7** and **8**. The molecular formula of $C_{25}H_{24}O_5$ was established via HRCIMS.

The ^{1}H NMR spectra of compounds **7** and **8** are virtually identical (see Table I). All of the spectral data supporting the structure given for inophyllum G-1 apply equally well for inophyllum G-2. In each of the ^{1}H NMR spectra, there are five intercoupled aromatic protons near δ 7.4, a sharp

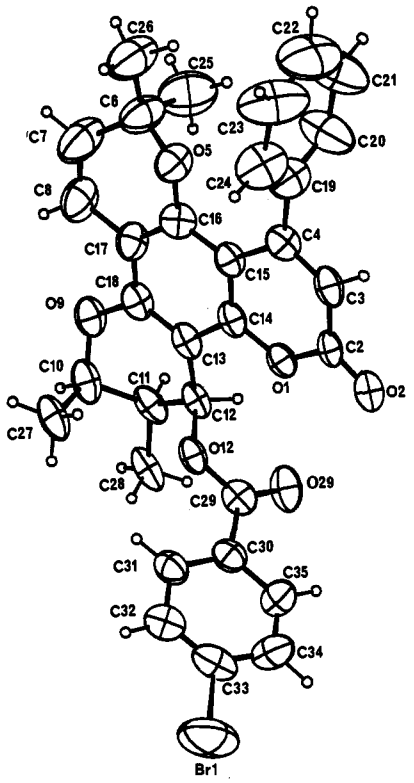

Figure 3. An ORTEP II drawing of the crystal structure for **1c**. Principal ellipses for non-hydrogen atoms are drawn at the 50% probability level. Hydrogen atoms are drawn as spheres of arbitrary size.

olefinic singlet at δ 6.0, five methine multiplets (three of which are doublets and two of which are doublets of doublets of quartets) ranging from δ 5.2 to 2.3, and four methyl groups (two of which are doublets and two of which are singlets). These spectra are very reminiscent of that of inophyllum A (**1a**). Notable differences between the ^{1}H NMR spectra of **1a** and **7** (and **8** as well) come in the form of an upfield shift of H-7 and H-8, the cis coupled olefinic doublets from δ 6.55 and 5.42 (J = 10.0 Hz) in **1a** to δ 4.23 and 2.46 (J = 5.7 Hz) in **7** (and **8**), as well as an upfield shift of the C-6 geminal methyl resonances. These spectral changes indicate the loss of the C-7/8 double bond in ring A. Since the molecular formulas of **1a**, **7**, and **8** are all identical, the loss of a double bond implies the presence of an additional ring. Because all of the differences between the spectra of **1a** and **7** (and **8** as well) involve ring-A protons, the presence of a 2-dimethylcyclopropane ring was proposed (see Figure 1). The observed 5.7-Hz coupling between the protons at δ 4.23 and 2.46 supports this proposal, as does their strong mutual NOE interactions between one another and the δ 1.05 methyl group. Two-dimensional heterocorrelation NMR data clearly confirm the presence of a cyclopropane ring with the quaternary gem dimethyl group located at C-7 (δ 13.0) rather than at C-6 (δ 76.8), as is the case for inophyllum A (**1a**).

Because inophyllums G-1 and G-2 (**7** and **8**) possess two widely separated centers of chirality, it is difficult to specify the absolute stereochemistry for these compounds. Since the chromanol ring protons show chemical shifts and coupling constants similar to those of inophyllum A (**1a**), it is assumed that the stereochemistry at this point is the same as that in **1a**. The protons on the cyclopropane ring are too distant from the chromanol ring protons to establish an NOE-based relationship between the two spin systems. Without an X-ray analysis, it is unclear whether the cyclopropane ring in **7** is α or β, but it is safe to say that **7** and **8** collectively represent the α-β pair. Both **7** and **8** were acetylated with acetic anhydride and pyridine to give monoacetates **7a** and **8a** whose proton NMR spectra displayed acetate signals at δ 2.15.

Table III. IC_{50} Values for Inophyllum Derivatives against HIV-1 Reverse Transciptase[a]

compound	IC_{50} (μM)
calophyllolide (**6**)	>100
inophyllum A (**1a**)	30
inophyllum B (**2a**)	0.038
inophyllum P (**2b**)	0.130
inophyllum D (**1b**)	11
inophyllum E (**3b**)	100
inophyllum C (**3a**)	10
calophyllic acid (**5a**)	>100
isocalophyllic acid (**5b**)	>100
inophyllum G-1 (**7**)	>100
inophyllum G-2 (**8**)	70
inophyllum A 4-bromobenzoate (**1c**)	46
inophyllum P acetate (**2c**)	>100
inophyllum B acetate (**2d**)	0.72
11,12-anhydroinophyllum P (**9**)	9.0
TIBO R82913[b]	0.70

[a] The scintillation proximity assay was used to determine IC_{50} values, as described in the Experimental Section and ref 14. [b] (+)-(5*S*)-4,5,6,7-Tetrahydro-9-chloro-5-methyl-6-(3-methyl-2-butenyl)imidazo[4,5,1-*jk*][1,4]benzodiazepine-2(1*H*)-thione.

Biological Activity of Inophyllum Derivatives

Structure–Activity Relationship. All of the pure compounds and their derivatives were evaluated for inhibitory activity against HIV-1 RT with a scintillation proximity assay developed by Amersham.[13,14] This procedure monitored the incorporation of radiolabeled nucleotides into the sequence of a biotinylated heteropolymeric template. After completion of the assay, the amount of incorporated nucleotides was quantified by binding the template to streptavidin-coated scintillant beads. Only the nucleotides which were part of the lengthened template were close enough to the scintillant bead to induce light emission. Among the compounds isolated from *C. inophyllum*, inophyllum B (**2a**) was consistently the most active compound followed closely by inophyllum P (**2b**), with IC_{50} values of 38 and 130 nM, respectively (Table III). Although the stereochemistry of the hydroxyl group at C-12 is not critical because **2a** and **2b** are both active at submicromolar concentrations, the presence of a carbonyl group at this position lowers the activity significantly; inophyllums C and E (**3a** and **3b**) are much less active or inactive. Furthermore, the 12-acetoxy derivatives **2c** and **2d** exhibited significantly lower activity compared to that of the 12-hydroxylated compounds **2a** and **2b**. Calophyllic and isocalophyllic acids (**5a** and **5b**), calophyllolide (**6**), and the novel inophyllums G-1 and G-2 (**7** and **8**) were all inactive. 11,12-Anhydroinophyllum P (**9**) had only minimal activity (9 μM); this activity was probably due to the presence of residual inophyllum P (**2b**) in this sample (as shown by NMR). The stereochemistries at C-10 (*R*) and C-11 (*S*) are important, as maximum activity is observed when the methyls at C-10 and C-11 of the chromanol ring are trans diaxial and the C-11 methyl group is α; for example, compare inophyllum B (**2a**) to inophyllum A (**1a**), and inophyllum P (**2b**) to inophyllum D (**1b**). From the published data on the calanolides, it appears that substitution of the phenyl at C-4 by a propyl group does not result in loss of activity.[9] In addition, the most active of the tested inophyllums (**2a**) has the identical stereo-

Table IV. Inhibition of HIV-1 Infectivity in Cell Culture by Inophyllums

compound	IC_{50} (μM) anti-HIV[a]	cellular toxicity[b]	selectivity index[c]
inophyllum B	1.4	55	39
inophyllum P	1.6	25	16
inophyllum A	ND[d]	33	NC[e]
inophyllum C	ND	18	NC
inophyllum D	ND	15	NC
inophyllum E	ND	6.2	NC
calophyllolide	ND	4.5	NC
TIBO R82913[f]	0.19	19	100

[a] Measured by RT quantitation 7 days after infection. [b] Host cells are Molt-4 cells; cytotoxicity measured by the XTT assay. [c] Cytotoxicity IC_{50}/anti-HIV IC_{50}. [d] ND, not determined. [e] NC, not calculated. [f] (+)-(5S)-4,5,6,7-Tetrahydro-9-chloro-5-methyl-6-(3-methyl-2-butenyl)imidazo[4,5,1-jk][1,4]benzodiazepine-2(1H)-thione.

chemistry as the most active calanolide described (calanolide A).[9]

Inophyllum B (2a) selectively inhibited the polymerase activity of HIV-1 RT; it was inactive against RNase H activity (data not shown). Furthermore, 2a is selective for HIV-1 RT relative to reverse transcriptases derived from HIV-2, avian myeloblastosis virus, and Moloney murine leukemia virus.[14] The inophyllums and calanolides appear to be somewhat different from other well-classified non-nucleoside inhibitors in that they inhibit certain mutants of HIV-1 RT in which the tyrosine at position 181 is altered.[9,10,14] For example, inophyllum B (2a) inhibited HIV-1 RT mutants in which tyrosine-181 was changed to either cysteine (IC_{50} = 160 nM) or leucine (IC_{50} = 410 nM).

Inhibition of HIV-1 in Cell Culture by Inophyllums and Tetrahydroimidazobenzodiazepinethione (TIBO R82913). Inophyllums B and P (2a and 2b) were evaluated for anti-HIV activity using a 7-day infectivity assay with RT quantification as the endpoint (Table IV). Cytotoxicity to host Molt-4 cells was quantified by the XTT assay.[15] Inophyllum B (2a) demonstrated anti-HIV activity with an IC_{50} value of 1.4 μM and was cytotoxic to host cells at 55 μM. Inophyllum P (2b) demonstrated a similar potency against HIV-1 but had a lower selective index (IC_{50} for cytotoxicity divided by IC_{50} for anti-HIV-1 activity). TIBO R82913, a non-nucleoside RT inhibitor, was approximately 7 times more potent than the inophyllums in this experiment. Neither of these compounds was active against HIV-2 in cell culture (data not shown).

The inophyllums inhibited HIV-1 infectivity in cell culture at concentrations approximately 40 times higher than the K_i for isolated RT. This difference may be due to poor cellular penetration and/or binding of inophyllum to components in the cell culture medium. Inophyllums demonstrated weak cytotoxicity to host cells, which was likely unrelated to inhibition of DNA polymerases. Inhibition of DNA polymerase α by inophyllum B (2a) was barely detectable at concentrations 1000 times the K_i for RT (data not shown). In addition, inophyllum derivatives inactive against RT also demonstrated cytotoxicity to Molt-4 cells at concentrations in the 50 μM range, suggesting that this effect is nonspecific and unrelated to RT inhibition.

Conclusion

Of the 11 metabolites isolated from *C. inophyllum*, reported for the first time are inophyllums P, G-1, and G-2 and the resolution of calophyllic and isocalophyllic acids. In addition, the absolute configurations of inophyllum A and the two calophyllic acids have been established and, hence, the absolute configuration of this whole series of compounds.

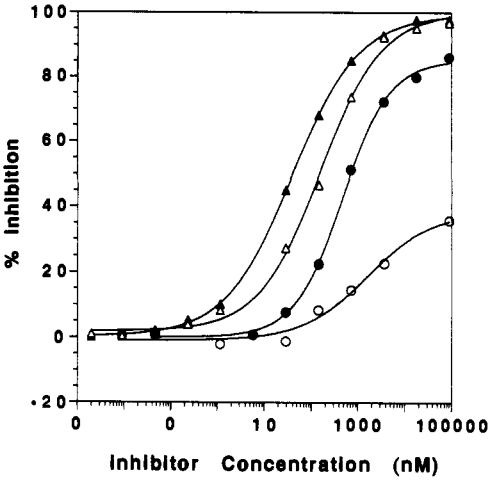

Figure 4. Inhibition of HIV-1 RT by (▲) inophyllum B, (△) inophyllum P, (●) inophyllum B acetate, and (○) inophyllum P acetate. HIV-1 RT activity was assayed as described in the Experimental Section and ref 14.

The considerable structural variation among these 11 natural products and the three semisynthetic analogs made preliminary SAR conclusions possible. If the earlier reports on the calanolides[9,10] are also taken into account, it appears that the stereochemistry of the methyl groups at C-10 and C-11 in inophyllums B and P and calanolide A is essential, the configuration of the hydroxyl at C-12 is somewhat less critical, and the nature of the substituent at C-4 is open to considerable variation.

The inophyllums are potent inhibitors of HIV-1 reverse transcriptase and have anti-HIV-1 activity in cell culture. The inophyllums[14] and calanolides appear to belong to a subclass of non-nucleoside RT inhibitors that are active against HIV-1 RT mutants resistant to most other non-nucleoside RT inhibitors.[14] Synthesis of derivatives of inophyllums and calanolides may lead to compounds with increased potency and selectivity.[19]

Experimental Section

General. IR spectra were recorded on a Nicolet Model 20 DXB FTIR spectrometer. All homonuclear and heteronuclear one- and two-dimensional NMR data were recorded on a Bruker AMX-400 spectrometer. LRCIMS and HRCIMS were performed on Finnigan 4610 and VG 70-VSE mass spectrometers, respectively. Pneumatically assisted electrospray mass spectra (ESMS) were recorded in CH_2Cl_2:MeOH (93:7) on a Perkin-Elmer Sciex APT III triple quadrupole. Analytical and preparative TLC were carried out on precoated silica gel G (Kiesel gel G254) and reversed-phase (Whatman KC18F) plates. A Rainin HPXL solvent delivery system equipped with a refractive index detector, Model 156, was used for HPLC separations, employing a Lichrosorb SI 60 (7-μm) column. UV spectra were recorded on a Beckman DV-7 spectrophotometer. X-ray data were collected on an Enraf Nonius CAD-4 diffractometer. Reagent grade chemicals (Fisher and Baker) were used.

Assay Procedure for the Determination of IC_{50}s on Inophyllums vs Reverse Transcriptase. The procedure employed a scintillation proximity assay kit (NK 8972) provided by Amersham and is described in more detail elsewhere.[14] Briefly, RNA-dependent DNA polymerase activity was monitored by using a 50-mer RNA heteropolymeric template annealed at its 5′-end to a biotinylated 17-mer DNA primer. The kit assay buffer was modified with respect to detergent such that its composition was 50 mM Tris-HCl, 80 mM KCl, 10 mM $MgCl_2$, 10 mM DTT, and 0.012% CHAPS at a pH of 8.0. The enzyme, substrate, and inhibitor concentrations in the assay were 2 nM RT, 39 nM template–primer, 75 nM dATP, dCTP, dGTP, and [methyl-1′,2′-

[3H]TTP (Amersham TRK-576), and serial dilutions of inhibitor encompassing a range of 90 μM–0.01 nM. A preincubation was performed prior to the assay wherein 10-μL aliquots of the working solutions of RT, template–primer, and inhibitor were mixed in a 96-well plate and placed in an incubator at 37 °C for 15 min. The dNTPs/[3H]TTP working solution was equilibrated to 37 °C in the same period. The assay was initiated by adding 70 μL of the dNTPs/[3H]TTP working solution to the 30-μL RT/template–primer/inhibitor mixture in each assay well. Assays were run for 10 min at 37 °C and terminated with 40 μL of 0.56 M EDTA at pH 8.0. The incorporated radiolabel was detected by adding 10 μL of a solution of streptavidin-coated scintillant beads in phosphate-buffered saline at pH 7.4 containing 10% v/v glycerol and 0.05% w/v sodium azide. This final mix was transferred to a T-tray (Pharmacia-Wallac), sealed, heated at 37 °C for 10 min, and then counted in a 1205 Betaplate liquid scintillation counter with its window settings fully open.

Cytotoxicity Testing. Cytotoxicity was assessed by the direct microscopic examination of trypan-blue-stained cells as well as the XTT assay as described by Weislow *et al.*[15] Molt-4 cells were plated at 3×10^4 cells/well containing the appropriate compound concentration. On day 7, the ability of the cells to metabolize XTT to its formazan dye was determined.

Antiviral Assay. Molt-4 cells (3×10^4) were infected with 50 tissue culture infectious doses of either HIV-1 or HIV-2. Compounds were added on day 0. The cultures were refed on days 1 and 4 with growth medium containing compounds at their initial concentrations. Cells were spun down on day 7, the time at which maximal viral RT production occurs, and the supernatant was quantified for RT by a microassay as described.[16,17]

Collection, Extraction, and Isolation. *A. fulica*. The snails, *A. fulica* (Bowditch) (90 individuals), were collected by hand at Beau Vallon, Mahe, Seychelles, during May of 1990. The frozen snails (150 g) were thawed, removed from their shells, and extracted sequentially with acetone and water. Removal of the solvent under reduced pressure and temperature gave 5.1- and 25.4-g extracts, respectively. The acetone extract which showed strong activity in the RT assay was adsorbed, applied to a column of RP-18 silica gel, and eluted with H_2O:MeOH (5:95). Fractions (15-mL each) were monitored by thin-layer chromatography (TLC) and pooled. Combined fractions (15–47, 284 mg) which were active in the RT inhibition assay were rechromatographed over a column of RP-18 silica gel using H_2O:CH_3CN (20:80) as the solvent system for elution. Fractions with the same TLC profile were combined to yield a greenish residue (101 mg). Finally, extensive silica gel PTLC (MeOH:CH_2Cl_2 10:90) and normal phase HPLC (EtOAc:hexane 25:75) of the active fraction yielded inophyllums A (**1a**, 1.1 mg), B (**2a**, 0.6 mg), C (**3a**, 2.1 mg), D (**1b**, 0.2 mg), and E (**3b**, 2.19 mg) and calophyllolide (**6**, 11.8 mg).

***C. inophyllum* Linn.** Leaves and twigs (0.5 kg) of *C. inophyllum* Linn. (Guttiferae) were collected in April 1992 near University Botanical Gardens in a surrounding forest by the Forest Research Institute of Malaysia, and a voucher sample (FE 3290) has been deposited in the Biotics collection at the University of Sussex, U.K. Extraction with MeOH:CH_2Cl_2 (1:1) by a cold percolation procedure yielded a dark green residue (46 g) which displayed strong RT inhibition activity and was triturated with CH_2Cl_2 to give a dark green oil (19.5 g). Silica gel (800 g) column chromatography of the CH_2Cl_2 soluble portion started with an EtOAc:hexane mixture (30:70), and then, increasing percentages of EtOAc in hexane were added. A total of 550 fractions (15-mL each) were collected and monitored by silica gel TLC. Like fractions were combined to give 20 individual fractions. Fractions 95–107 (1.05 g) after RP-18 column chromatography using CH_3CN as the solvent for elution yielded several fractions. Fractions 22–48 (108 mg) from this column after silica gel HPLC (EtOAc:hexane 25:75) furnished calophyllolide (**6**, 0.094 g).

The combined residue of fractions 108–134 (0.925 g) from the silica gel column was chromatographed on a column of RP-18 silica gel (H_2O:CH_3CN 5:95) to give several subfractions which after silica gel HPLC (EtOAc:hexane 25:75) gave inophyllum A (**1a**, 0.405 g) and **6** (0.022 g). Fractions 135–180 (0.912 g) after RP-18 column (CH_3CN) and silica gel PTLC gave additional inophyllum A (0.437 g) as a major component. A minor subfraction (0.018 g) from the RP-18 column, which was crystalline and appeared homogeneous by TLC, was resolved using silica gel HPLC (EtOAc:hexane 25:75) to yield inophyllums B (**2a**, 0.007 g) and P (**2b**, 0.008 g). Another minor subfraction (0.022 g) which also appeared to be a single compound by TLC was separated by normal phase HPLC (EtOAc:hexane 25:75) to yield inophyllums G-1 (**7**, 0.009 g) and G-2 (**8**, 0.008 g). Fractions 191–235 (0.399 g) from the silica gel column after repeated PTLC using silica gel (MeOH:CH_2Cl_2 3:97 and EtOAc:hexane 35:65) yielded two compounds which were identified as inophyllums C (**3a**, 0.099 g) and D (**1b**, 0.032 g). Fractions 236–310 (0.638 g) were purified by silica gel PTLC (EtOAc:hexane 35:65) to afford inophyllum E (**3b**, 0.277 g) and also an additional amount (0.073 g) of inophyllum C (**3a**). A polar residue from fractions 404–466 (0.431 g) afforded calophyllic and isocalophyllic acids (**5a**, 0.098 g, and **5b**, 0.116 g) after silica gel PTLC (EtOAc:hexane 1:1) and crystallization from hot EtOAc.

Inophyllum P (2b): white powder, $[\alpha]_D$ +19.8° ($CHCl_3$, c 0.8); UV λ_{max} (MeOH) 207, 235, 280, 286, 334 nm; IR (KBr) ν_{max} 3435, 2930, 2860, 1719, 1640, 1590, 1565, 765, 703 cm^{-1}; ^1H NMR (see Table I); ^{13}C NMR (see Table II); ESMS $(M + H)^+$ m/z 405; HRCIMS (methane) obsd 405.1732 $(M + H^+)$, calcd for $C_{25}H_{25}O_5$ 405.1702.

11,12-Anhydroinophyllum P (9): $[\alpha]_D$ +34.9° ($CHCl_3$, c 0.25); UV λ_{max} (MeOH) 207, 247, 284, 345 nm; IR (KBr) ν_{max} 2861, 1719, 1659, 765, 703 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.38 (m, 3H), 7.24 (m, 2H), 6.64 (b s, 1H), 6.54 (d, J = 10 Hz, 1H), 5.95 (s, 1H), 5.39 (d, J = 10.0 Hz, 1H), 4.91 (q, 1H), 1.88 (s, 3H), 1.41 (d, J = 7.0 Hz, 3H), 0.96 (s, 3H), 0.93 (s, 3H); HRCIMS (methane) obsd 386.1518, calcd for $C_{25}H_{22}O_4$ 386.1518.

Acetylation of Inophyllum P (2b). Inophyllum P (2.5 mg) was dissolved in acetic anhydride:pyridine (1:1, 0.5 mL) and the reaction mixture left at room temperature for 12 h. Water was added, and the mixture was extracted with chloroform. Monoacetate **2c** was purified by silica gel PTLC with an ethyl acetate:hexane mixture (10:90) as a colorless powder (2.4 mg): $[\alpha]_D$ +4.9° ($CHCl_3$, c 0.23); UV λ_{max} (MeOH) 208, 231, 284, 328 nm; IR (KBr) ν_{max} 1748, 1731, 765, 703 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.36 (m, 3H), 7.29 (m, 2H), 6.52 (d, J = 10 Hz, 1H), 6.46 (d, J = 3.4 Hz, 1H), 5.96 (s, 1H), 5.35 (d, J = 10.0 Hz, 1H), 4.13 (m, 1H), 2.12 (s, 3H), 1.94 (m, 1H), 1. 44 (d, J = 6.3 Hz, 3H), 1.05 (d, J = 6.9 Hz, 3H), 0.95 (s, 3H), 0.94 (s, 3H); ESMS $(M + H)^+$ m/z 447; HRCIMS (methane) obsd 446.1726, calcd for $C_{27}H_{26}O_6$ 446.1729.

Acetylation of Inophyllum B (2a). Inophyllum B (3.5 mg) was dissolved in acetic anhydride:pyridine (1:1, 0.7 mL) and the reaction mixture left at room temperature for 12 h. Water was added, and the mixture was extracted with chloroform. The colorless gum was purified by silica gel PTLC with an ethyl acetate:hexane (10:90) mixture to yield and amorphous powder (**2d**, 3.5 mg): UV λ_{max} (MeOH) 207, 232, 285, 329 nm; IR (KBr) ν_{max} 1747, 1732, 765, 704 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.36 (m, 3H), 7.24 (m, 2H), 6.53 (d, J = 10 Hz), 6.05 (d, J = 5.7 Hz), 5.96 (s, 1H), 5.36 (d, J = 10 Hz, 1H), 4.20 (m, 1H), 2.16 (s, 3H), 2.12 (m, 1H), 1.46 (d, J = 6.6 Hz, 3H), 1.09 (d, J = 7.1 Hz, 3H), 0.96 (s, 3H), 0.93 (s, 3H); ESMS $(M + H)^+$ m/z 447; HRCIMS (methane) obsd 446.1756, calcd for $C_{27}H_{26}O_6$.

Reduction of Inophyllum C (3a). Inophyllum C (25 mg) in methanol (5 mL) was treated with sodium borohydride (50 mg). The mixture was stirred for 3 h, the excess hydride was destroyed with water, and the product was extracted with methylene chloride. The gum obtained on evaporation was separated on a silica gel plate with ethyl acetate:hexane (30:70) into two compounds which were identified as inophyllum B (**2a**, 11 mg) and its 12-epimer, inophyllum P (**2b**, 8 mg), by direct comparison with the natural products.

Reduction of Inophyllum E (3b). Inophyllum E (25 mg) in methanol (5 mL) was treated with sodium borohydride (50 mg), and the reaction was worked up as discussed above. The two alcohols obtained were inophyllums A (**1a**, 13 mg) and D (**1b**, 7 mg), and their identities were confirmed by direct comparison with the natural products.

Benzoylation of Inophyllum A (1a). To a solution of inophyllum A (50 mg) in 5 mL of acetonitrile was added 4-(*N*,*N*-dimethylamino)pyridine (50 mg) and 4-bromobenzoyl chloride (150 mg). The resulting white suspension was stirred at room temperature for 3 h. The reaction mixture was diluted with cold

0.1 N HCl and extracted with ether (2 × 50 mL). The ether layer was washed successively with cold 0.1 N HCl (twice), saturated aqueous $NaHCO_3$, water (twice), and brine and then dried over $MgSO_4$. Evaporation of the solvent afforded a residue that was purified by silica gel PTLC with ethyl acetate:hexane (30:70) to yield an amorphous powder (1c, 52 mg) which crystallized from ethyl acetate as colorless needles: $[\alpha]_D$ +5.1° ($CHCl_3$, c 2.6); UV λ_{max} (MeOH) 206, 239, 285, 328 nm; ^1H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, J = 8.6 Hz, 2H), 7.56 (d, J = 8.6 Hz, 2H), 7.35 (m, 3H), 7.21 (m, 2H), 6.66 (d, J = 4.4 Hz, 1H), 6.55 (d, J = 10.0 Hz, 1H), 5.90 (s, 1H), 5.40 (d, J = 10.0 Hz, 1H), 4.51 (dq, J = 6.7, 3.3 Hz, 1H), 2.61 (m, 1H), 1.47 (d, J = 6.8 Hz, 3H), 1.10 (d, J = 7.1 Hz, 3H), 0.96 (s, 3H), 0.95 (s, 3H); LRCIMS m/z 586; HRCIMS obsd 586.0919, calcd for $C_{32}H_{27}BrO_6$.

Inophyllum G-1 (7): colorless solid, $[\alpha]_D$ +174.2° ($CHCl_3$, c 0.3); UV λ_{max} (MeOH) 216, 219, 315, 333 nm; IR (KBr) 3600–3200, 3100–3000, 2850, 1719, 1700, 1700, 1142, 1097, 853, 766, 736, 700 cm^{-1}; ^1H NMR (see Table I); ^{13}C NMR (see Table II); LRCIMS m/z 404; HRCIMS obsd 404.1610, calcd for $C_{25}H_{24}O_5$.

Acetylation of G-1. Inophyllum G-1 (1 mg) was dissolved in acetic acid anhydride:pyridine (1:1, 0.25 mL) and the reaction mixture left at room temperature for 12 h. The reagents were evaporated to yield inophyllum G-1 monoacetate (**7a**, 1 mg): ^1H NMR (400 MHz, $CDCl_3$) δ 7.41 (m, 3H), 7.31 (m, 2H), 6.34 (d, J = 5.2 Hz), 6.01 (s, 1H), 4.27 (m, 1H), 4.23 (d, J = 5.7 Hz), 2.66 (m, 1H), 2.46 (d, J = 5.7 Hz), 1.46 (d, J = 6.6 Hz, 3H), 1.22 (d, J = 7.0 Hz, 3H), 1.05 (s, 3H), 0.71 (s, 3H); LRCIMS m/z 446; HRCIMS obsd 446.1730, calcd for $C_{27}H_{26}O_6$.

Inophyllum G-2 (8): colorless solid, $[\alpha]_D$ −49.1° ($CHCl_3$, c 0.11); UV λ_{max} (MeOH) 215, 217, 224, 335 nm; IR (KBr) 3600–3200, 3100–3000, 3000–2800, 1718, 1699, 1142, 1095, 855, 770, 741, 701 cm^{-1}; ^1H NMR (see Table I); ^{13}C NMR (see Table II): LRCIMS m/z 404; HRCIMS obsd 404.1610, calcd for $C_{25}H_{24}O_5$.

Acetylation of G-2. Inophyllum G-2 (1 mg) was dissolved in acetic anhydride:pyridine (1:1, 0.25 mL), and the reaction mixture was left at room temperature for 12 h. The reagents were evaporated to yield inophyllum G-2 monoacetate (**8a**, 1 mg): ^1H NMR (400 MHz, $CDCl_3$) δ 7.42 (m, 3H), 7.32 (m, 2H), 6.35 (d, J = 5.4 Hz, 1H), 6.01 (s, 1H), 4.38 (m, 1H), 4.20 (d, J = 5.7 Hz), 2.55 (m, 1H), 2.42 (d, J = 5.7 Hz, 1H), 1.45 (d, J = 6.6 Hz, 3H), 1.18 (d, J = 7.1 Hz, 3H), 1.05 (s, 3H), 0.73 (s, 3H); LRCIMS m/z 446; HRCIMS obsd 446.1722, calcd for $C_{27}H_{26}O_6$.

X-ray Structure Determination of Inophyllum A 4-Bromobenzoate. Crystal Data for 1c: $C_{32}H_{27}BrO_6$, M_r = 587.48, tan plates, orthorhombic, space group $P2_12_12_1$, a = 6.558(1) Å, b = 13.383(1) Å, c = 32.261(2) Å, V = 2844.2(3) Å^3, Z = 4, d_{calc} = 1.369 g cm^{-3}, μ = 23.084 cm^{-1}, (Cu Kα, λ = 1.5484 Å), T = 223 K. An octant of intensity data $2\theta \le 111°$, $0 \le h \le 7$, $0 \le k \le 15$, $0 \le l \le 37$ and their Friedel mates were collected using variable-speed ω–2θ scans. Data were corrected for Lorentz and polarization factors, for absorption, and for isotropic intensity decay. The structure was solved by direct methods and refined with anisotropic displacement parameters for non-hydrogen atoms. Hydrogen-atom positions were determined from difference Fourier maps but were held fixed at geometrically calculated positions in the final refinement with isotropic temperature factors assigned 1.3 (Beq) of the attached atom. Full matrix least-squares refinement (on F) converged (max Δ/σ = 0.01) to conventional crystallographic residuals R = 0.059 and R_w = 0.084 for 2349 observations [$I \ge 3\sigma(I)$]. There were 352 variables and the goodness of fit was 1.423. A final difference Fourier map was featureless; maximum residual densities were near the bromine atom (0.642 and −0.123 eÅ^{-3}). The absolute configuration was assigned on the basis of the R factor ratio test. Each enantiomer was refined separately, and the resulting ratio of 1.083 for weighted residuals is significant at the 99.99% confidence level. This assignment was confirmed by an enantiopole refinement and further corroborated by examination of the Friedel pairs most affected by anomalous dispersion effects. Neutral atom scattering factors and values for the anamolous dispersion terms were taken from the International Tables for X-ray Crystallography.[18]

Acknowledgment. We thank Dr. Brad Carte and Mary-Kay Harper for the collection of *A. fulica* and Drs. Gary Rosenberg and Kenneth Emberton of the Malacology Department of the Academy of Natural Sciences, Philadelphia, PA, for identification of *A. fulica*. We also thank Dr. Robert Thomas of Biotics Ltd. (U.K.) for provision of *C. inophyllum* from Malaysia.

Supplementary Material Available: Fractional atomic coordinates, anisotropic displacement parameters, and bond distances and angles for compound 1c (7 pages); a table of observed and calculated structure factors (18 pages). Ordering information is given on any current masthead page.

References

(1) Fischl, M. A.; Richman, D. D.; Grieco, M. H.; Gottlieb, M. S.; Volberding, P. A.; Laskin, O. L.; Leedom, J. M.; Groopman, J. E.; Mildvan, D.; Schoolery, R. T.; Jackson, G. G.; Durack, D. T.; King, D. The AZT collaborative working group. The Efficacy of Azidothymidine (AZT) in the Treatment of Patients with AIDS and AIDS-Related Complex. *N. Engl. J. Med.* **1987**, *317*, 185–191.
(2) Butler, K. M.; Husson, R. N.; Balis, F. M.; Brouwers, P.; Eddy, J.; El-Amin, D.; Gress, J.; Hawkins, M.; Jarosinsky, P.; Moss, H.; Poplack, D.; Santacroce, S.; Venzon, D.; Wiener, L.; Wolters, P.; Pizzo, P. A. Dideoxyinosine in Children with Symptomatic Human Immunodeficiency Virus Infection. *N. Engl. J. Med.* **1991**, *324*, 137–144.
(3) Larder, B. A.; Kemp, S. D. Multiple Mutations in HIV-1 Reverse Transcriptase Confer High-Level Resistance to Zidovudine (AZT). *Science* **1989**, *246*, 1155–1158.
(4) St. Clair, M. H.; Martin, J. L.; Tudor-Williams, G.; Bach, M. C.; Vavro, C. L.; King, D. M.; Kellam, P.; Kemp, S. D.; Larder, B. A. Resistance to ddI and Sensitivity to AZT Induced by a Mutation in HIV-1 Reverse Transcriptase. *Science* **1991**, *253*, 1557–1559.
(5) Kawazu, K.; Ohigashi, H.; Mitsui, T. The Piscicidial Constituents of *Calophyllum inophyllum*. *Tetrahedron Lett.* **1968**, *19*, 2383–2385.
(6) Kawazu, K.; Ohigashi, H.; Takahashi, N.; Mitsui, T. Piscicidial Constituents of *Calophyllum inophyllum*. *Bull. Inst. Chem. Res., Kyoto Univ.* **1972**, *50*, 160–167.
(7) Gunasekera, S. P.; Jayatilake, G. S.; Selliah, S. S.; Sultanbawa, M. U. S. Chemical Investigation of Ceylonese Plants. Part 27. Extractives of *Calophyllum cuneifolium* Thw. and *Calophyllum soulattri*. *J. Chem. Soc., Perkins Trans. I* **1977**, 1505–1511.
(8) Ponglux, D.; Wongseripipatana, S.; Phadungcharoen, T.; Ruangrungsri, N.; Likhitwitayawuid, K. Medicinal Plants. Medicinal Plants Exhibition Committee, The Forest Princess Chulabhorn Science Congress; International Congress on Natural Products: Bangkok, Thailand, Dec 10–13, 1987; p 57.
(9) Kashman, Y.; Gustafson, K. R.; Fuller, R. W.; Cardellina, J. H.; McMahon, J. B.; Currens, M. J.; Buckheit, R. W., Jr.; Hughes, S. H.; Cragg, G. M.; Boyd, M. R. The Calanolides, a Novel HIV-Inhibitory Class of Coumarin Derivatives from the Tropical Rainforest Tree, *Calophyllum lanigerum*. *J. Med. Chem.* **1992**, *35*, 2735–2743.
(10) Boyer, P. L.; Currens, M. J.; McMohan, J. B.; Boyd, M. R.; Hughes, S. H. Analysis of Nonnucleoside Drug-Resistant Variants of Human Immunodeficiency Virus Type 1 Reverse Transcriptase. *J. Virol.* **1993**, *67*, 2412–2420.
(11) Mead, A. R. Economic Status. In *The Giant African Snail: A Problem In Economic Malacology*. The University of Chicago Press: Chicago, 1961; p 42.
(12) Patil, A. D.; Haltiwanger, C.; Westley, J. W.; Eggleston, D. S. The Structures of Calophyllic Acid and Iso-calophyllic Acid. *Acta Crystallogr.*, submitted.
(13) Bosworth, N.; Towers, P. Scintillation Proximity Assay. *Nature* **1989**, *341*, 167–168.
(14) Taylor, P. B.; Culp, J. S.; Debouck, C.; Johnson, R. K.; Patil, A. D.; Woolf, D. J.; Bartus, H. R.; Brooks, I.; Hertzberg, R. P. Kinetic and Mutational Analysis of HIV-1 Reverse Transcriptase Inhibition by Inophyllums, a Novel Class of Non-Nucleoside Inhibitors, Using a Scintillation Proximity Assay. *J. Biol. Chem.* **1993**, in press.
(15) Weislow, O. S.; Kiser, R.; Fine, D. L.; Bader, J.; Shoemaker, R. H.; Boyd, M. R. New Soluble-Formazan Assay for HIV-1 Cytopathic Effects: Application to High-Flux Screening of Synthetic and Natural Products for AIDS-Antiviral Activity. *J. Nat. Cancer Inst.* **1989**, *81*, 577–586.
(16) Goff, S.; Traktman, P.; Baltimore, D. Isolation and Properties of Moloney Murine Leukemia Virus Mutants: Use of a Rapid Assay for Release of Virion Reverse Transcriptase. *J. Virol.* **1981**, *38*, 239–248.
(17) Willey, R. L.; Smith, D. H.; Lasky, L. A.; Theodore, T. S.; Earl, P. L.; Moss, B.; Capon, D. J.; Martin, M. A. In Vitro Mutagenesis Identifies a Region within the Envelope Gene of the HUman Immunodeficiency Virus That Is Critical for Infectivity. *J. Virol.* **1988**, *62*, 139–147.
(18) *International Tables for X-ray Crystallography*. Kluwer Academic Publishers: Dordrecht, The Netherlands, 1974; Vol. IV.
(19) Chenera, B.; West, M. L.; Finkelstein, J. P.; Dreyer, G. B. Total Synthesis of (±)-Calanolide A, a Non-Nucleoside Inhibitor of HIV-1 Reverse Transcriptase. *J. Org. Chem.* **1993**, *58*, 5605–5606.