Isolation, Structure, Absolute Stereochemistry, and HIV-1 Integrase Inhibitory Activity of Integrasone, a Novel Fungal Polyketide

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HIV-1 integrase is a critical enzyme for replication of HIV, and its inhibition is one of the most promising new drug targets for anti-retroviral therapy with potentially significant advantages over existing therapies. In this Note, the isolation, structure elucidation, and absolute stereochemistry of integrasone, a novel polyketide, derived from an unidentified sterile mycelium have been described. This bicyclic dihydroxy epoxide lactone inhibited the strand transfer reaction of HIV-1 integrase with an IC_{50} of 41 μM.

HIV-1 integrase is one of the three enzymes that are critical for viral replication. It catalyzes three essential steps that include assembly, endonucleolytic cleavage (3′-end processing) of the viral DNA, and strand transfer of the viral DNA into the host cell DNA.[1−4] The inhibitors of the other two key enzymes, reverse transcriptase and protease, have led to many clinical agents that continue to have enormous impact on the control of the spread of HIV-1 infection. However the emergence of multi-drug-resistant virus, particularly now even in drug-naïve patients, has become a serious cause for concern, and anti-HIV-1 therapy with a new mode of action is needed. The absence of HIV-1 integrase in the host cells and its essential requirement for viral replication reveal this as a very good target for the development of a nontoxic anti-retroviral therapeutic agent. Recently much progress has been made in identification of inhibitors of this enzyme.[5−9]

Natural products have been a very good source of novel inhibitors for many biological targets, most importantly anti-infective targets. Screening using recombinant HIV-1 integrase (50−220 AA) led to the discovery of a number of natural product inhibitors exemplified by integric acid[10,11] and cytosporic acid.[12] Continued screening with strand transfer assay format[13] led to the discovery of a novel polyketide trivially named integrasone (**1**), from a unidentified sterile (nonreproductive, i.e., without any reproductive progagules, like spores) mycelium. The isolation, structure elucidation, relative stereochemistry, and HIV-1 inhibitory activity of this bicyclic dihydroxy epoxy lactone are described herein.
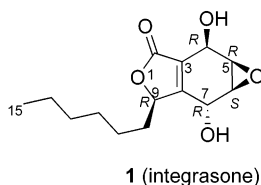
1 (integrasone)

Isolation. Integrasone was produced by an unidentified fungus (MF6836) that was grown on a vermiculite-based

Table 1. ^{1}H and ^{13}C NMR Assignments of Integrasone (**1**) in CD₃OD

position	δ_C	mult	δ_H (mult, *J* in Hz[a])	HMBC
2	173.3	qC		H-5, 9
3	126.0	qC		H-4, 5,7, 9
4	62.2	CH	4.80, dt, 3.0, 1.0	H-5, 6
5	55.7	CH	3.60, dt, 1.0, 3.5	H-6, 7
6	57.1	CH	3.48, dd, 3.5, 1.5	H-5, 7
7	62.1	CH	4.68, brs	H-5
8	162.0	qC		H-4, 6, 7, 9, 10
9	84.4	CH	5.0, ddd, 8.5, 3.0, 1.0	H-6, 7, 10, 11
10	34.2	CH₂	2.11, dddd, 14.0, 6.5, 5, 3.5	H-9
			1.60, dddd, 15.0, 10, 8.5, 4.5	
11	25.9	CH₂	1.40, m	H-9, 10
			1.48, m	
12	30.1	CH₂	1.36, m	H-10
13	23.6	CH₂	1.32, m	H-14, 15
14	32.8	CH₂	1.30, m	H-15
15	14.4	CH₃	0.91, t, 7.0	H-14

[a] All *J* values were measured after resolution enhancement.

solid media AD2.[14] Gel permeation chromatography of the methyl ethyl ketone extract of the sterile mycelial growth on Sephadex LH 20 followed by reversed-phase HPLC afforded integrasone (**1**) as an amorphous powder (1.8 g/L).

Structure. High-resolution ESIFTMS analysis of **1** produced a molecular formula of $C_{14}H_{20}O_5$, indicating the presence of five degrees of unsaturation that was corroborated by the ^{13}C NMR spectral analysis. The UV spectrum showed a single maximum at 213 nm ($\epsilon = 9570$), and the IR spectrum indicated the presence of hydroxy groups (3358 cm⁻¹) and a hydrogen-bonded conjugated lactone (1718 cm⁻¹). The ^{13}C NMR spectrum (125 MHz) of **1** in CD₃OD showed 14 carbon signals (Table 1). The DEPT spectrum revealed the presence of a lactone carbonyl (δ_C 173.3), two tetrasubstituted olefinic carbon atoms (δ_C 126.0, 162.0), a deshielded oxymethine (δ_C 84.4), and four relatively shielded oxymethines, five methylenes, and a methyl group. The presence of these groups was supported by the corresponding signals in the 500 MHz ^{1}H NMR spectrum. An HMQC experiment helped in an unambiguous assignment of proton connectivity to the respective proton-bearing carbons (Table 1). The COSY spectrum suggested two contiguous fragments consisting of four shielded oxymethines that were connected together in a sequence forming

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a contiguous fragment C4−C7 and the alkyl chain fragment C9−C15 that contained the remaining oxymethine. H-9 appeared as a doublet of doublets of doublets (δ_H 5.0, $J = 8.5, 3.0, 1.0$ Hz) and exhibited a cross-peak to H-4 (δ_H 4.80, dd, $J = 3.0, 1.0$) due to five-bond coupling ($J = 1$ Hz) and a cross-peak to H-7 due to four-bond coupling (exact J value must be less the 1 Hz and could not be determined, signal was just broadened, vide infra) through an olefin. The latter proton appeared as a broad singlet at δ_H 4.68 and exhibited two additional albeit very small couplings: a vicinal coupling with H-6 ($J = 1.5$ Hz) and a coupling with H-5 ($J = 1$ Hz). The identities of the two fragments were further corroborated by a TOCSY experiment. Finally, the structure of these fragments and the connectivity to each other and to the remaining carbons were established by an HMBC experiment recorded at $^nJ_{XH} = 7$ Hz. The H-4 produced strong HMBC correlations to both olefinic carbons C-3 (δ_C 126.0) and C-8 (δ_C 162.0). The low-field shift of C-8 suggested that it is likely to be β to the lactone carbonyl C-2 (δ_C 173.3), which showed a weak four-bond HMBC correlation to the epoxy methine H-5 and a strong correlation to oxymethine H-9, thus establishing the lactone ring. Both olefinic carbons (C-3 and C-8) showed HMBC correlations to H-7 and H-9, thus confirming the fusion of the six-membered dihydroxy epoxy ring with the five-membered lactone ring.

Stereochemistry. The relative stereochemistry of **1** was deduced by scalar couplings, NOESY experiment, and Dreiding models. The epoxide protons H-5 and H-6 exhibited a J value of 3.5 Hz due to couplings to each other. H-4 showed a coupling of ∼3.0 Hz with H-5, a weak *W*-coupling ($J = ∼1.0$ Hz) with H-6, and five-bond coupling ($J = ∼1.0$ Hz) with H-9, establishing a *cis*-relationship of H-4, H-5, and H-6. This assignment was further supported by the strong NOESY correlations of H-4 (α-orientation in planar conformation) and H-5, and H-5 and H-6, and weak correlations of H-6 and H-7. H-7 appeared as a broad singlet and exhibited no significant couplings, indicating that it was at ∼90° with respect to H-6, suggesting that it must be in a β-orientation in the planar conformation. The H-7 exhibited stronger NOESY correlation to the methylene protons at C-10, placing the chain in the same β-plane as H-7, and weaker NOESY correlation with H-9, favoring the C-9 stereochemistry as drawn.

The absolute configuration of integrasone was determined from the analysis of the two sets of mono MTPA ester derivatives **2**−**5**. Reaction of integrasone with (*S*)- and (*R*)-MTPA chlorides gave a mixture of 4- and 7-(*R*)-MTPA esters **2** and **3** and 4- and 7-(*S*)-MTPA esters **4** and **5**, respectively, and small amounts of respective diesters. The monoester mixtures were separated from the diester, and the unreacted starting material was separated by reversed-phase HPLC. However, **2** from **3**, and **4** from **5**, could not be separated and were used as a mixture for the analysis. The ^{1}H NMR spectrum of **2**−**5** was thoroughly assigned by the COSY and the NOESY spectroscopic analysis of the two mixtures.[15] The $\Delta\delta$ ($S − R$) of 4- and 7-esters were calculated, and the values are shown in Figure 1 (A and B). The 7-MTPA ester derivative B led to the stereochemical assignment of all five asymmetric centers as 4*R*, 5*R*, 6*S*, 7*R*, and 9*R*. While the 4-MTPA ester derivative A (Figure 1) supported the stereochemical assignment, the data could not be used independently for stereochemical assignment due to lack of the protons on the left side of the molecule, yielding only a weak shielding effect of the protons on the right-hand side of the molecule. On the basis
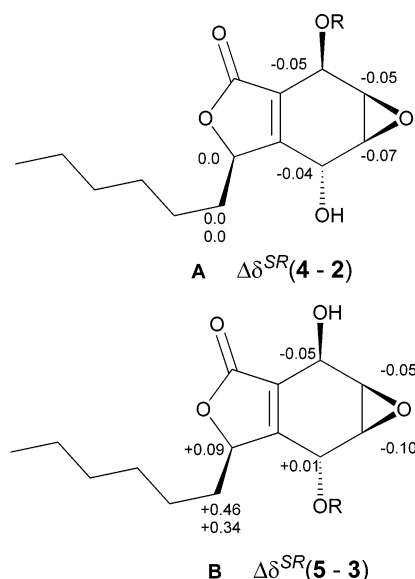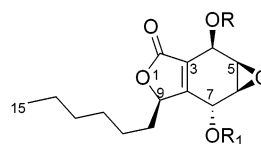

Figure 1. $\Delta\delta$ ($S − R$) of (A) 4- and (B) 7-MTPA esters of integrasone (**1**).

of the spectroscopic data, structure **1** was proposed for integrasone.

2 [R = (*R*)-MTPA, R$_1$ = H]
3 [R = H, R$_1$ = (*R*)-MTPA]
4 [R = (*S*)-MTPA, R$_1$ = H]
5 [R = H, R$_1$= (*S*)-MTPA]

A plausible biogenesis of integrasone may involve a 14-carbon polyketide chain from seven acetate units with appropriate reductions, oxidations, and cyclizations. STN substructure searches of integrasone did not afford directly similar compounds except for a list of analogous 1,4-dihydroxy aromatic lactones, indicating that integrasone may be a biosynthetic precursor of such aromatic compounds.

HIV-1 Integrase Activity. Integrasone inhibited the strand transfer reaction[13] of HIV-1 integrase with an IC$_{50}$ of 41 μM, and the aliphatic chain may play a significant role in the activity, as was observed in the case of integric acid (**6**).[10,11]

6 (integric acid)
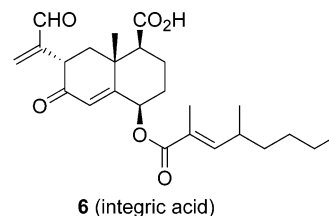
In summary, we have described the isolation, structure, and stereochemistry of integrasone, a novel 14-carbon polyketide, which is a modest inhibitor of the strand transfer reaction of HIV-1 integrase.

Experimental Section

General Experimental Procedures. All NMR spectra were recorded on Varian Inova 500 MHz instruments operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C nuclei. LC-MS

was performed on a Thermo Quest LCQ instrument using electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI). High-resolution mass spectral analyses were performed on a Thermo Quest FTMS using electrospray ionization. For column chromatography silica gel H (E. Merck 60–200 mesh) was used. An HP1100 by Agilent Corporation was used for analytical HPLC. Junlon (Nihon Junyaku Co., Ltd, Tokyo, Japan) is a cross-linked poly(acrylic acid) added to the fermentation as an inert emulsifying and thickening agent to prevent mycelial clumps and pellets.

Fermentation and Production of Integrasone (1). An unidentified fungal isolate (MF6836) was grown on a seed medium consisting of yeast extract (4 g), malt extract (8 g), glucose (4 g), and Junlon (1.5 g) in 1 L of distilled water at pH 7.0. After 7 days, it was transferred to a vermiculite-based AD2 production media consisting of (g/L) glucose (autoclaved separately) (150), glycerol (20), yeast extract (4), $NaNO_3$ (1), monosodium glutamate (3), Na_2HPO_4 (0.5), $MgSO_4 \cdot 7H_2O$ (1), trace elements (1 mL), and $CaCO_3$ (8), pH 7. The trace elements solution contains (g/L) $FeCl_3 \cdot 6H2O$ (5.8), $MnSO_4 \cdot H_2O$ (0.1), $CoCl_2 \cdot 6H_2O$ (0.02), $CuSO_4 \cdot 5H_2O$ (0.015), $Na_2MoO_4 \cdot 2H_2O$ (0.012), $ZnCl_2$ (0.02), $SnCl_2 \cdot 2H_2O$ (0.005), H_3BO_3 (0.01), KCl (0.02), and HCl (2 mL). The autoclaved solution (220 mL) containing AD2 medium was added to 675 cm^3 (by volume) previously sterilized large-particle vermiculite in a 2 L roller bottle incubated at 22 °C for 25 days and was extracted with 250 mL of methyl ethyl ketone.

Isolation of Integrasone. A 30 mL aliquot of the methyl ethyl ketone (MEK) extract of the sterile mycelial growth (MF6836) was concentrated under reduced pressure and lyophilized to yield 85 mg of crude material. Dried MEK extract was dissolved in 20 mL of MeOH and charged onto a 500 mL Sephadex LH20 column and eluted with MeOH at a flow rate of 2 mL/min. Twenty milliliters of each fraction were collected. Activity eluted in fractions 16–20 (0.6–0.8 cv). These fractions were pooled to give 50 mg of a residue. This was further purified by preparative reversed-phase HPLC using a Zorbax RX C-8 (21 × 250 mm) column eluting with 40% aqueous acetonitrile containing 0.1% TFA at a flow rate of 8 mL/min. Fractions eluting at 15 min contained all activity. It was concentrated and lyophilized to give 16 mg (1.8 g/L) of integrasone (1) as a colorless amorphous powder. HPLC t_R = 9.1 min (Zorbax RX C-8, 4.6 × 250 mm, 1 mL/min, 15 min gradient of 20 to 90% aqueous acetonitrile containing 0.1% TFA): $[\alpha]^{25}_D$ +16.7° (c 1.5, MeOH); UV (MeOH) λ_{max} 213 (ϵ = 9570) nm; IR (ZnSe) ν_{max} 3358, 2924, 2858, 1718, 1624, 1543, 1441, 1379, 1334, 1282, 1160, 1129, 1091, 1036, 1057, 984, 930 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1); ESIMS (m/z) 269 [M + H]$^+$, 251 [M − H$_2$O + H]$^+$, 233 [M − 2H$_2$O + H]$^+$; HRESIFTMS (m/z) 269.1394 (M + H, calcd for $C_{14}H_{20}O_5$+H, 269.1389).

Integrasone 4- and 7-(R)-MTPA Esters (2 and 3). A solution of (S)-MTPA chloride (2 mg) in 10 μL of methylene chloride was added to a solution of **1** (1 mg), pyridine (50 μL), and DMAP (3 mg) in 100 μL of methylene chloride. The solution was stirred at room temperature overnight. The progress of the reaction was monitored by reversed-phase HPLC. A significant amount of unreacted starting material was present in the reaction. An additional 4 mg of aliquot of (S)-MTPA chloride in 20 μL of methylene chloride was added, and the reaction was stirred for 4 h. The reaction was quenched by addition of 50 μL of water. One milliliter of EtOAc was added, layers were separated, and the organic layer was washed with 10% aqueous citric acid and water, dried (NaSO$_4$), concentrated to dryness, and chromatographed by reversed-phase HPLC (Zorbax RX C-8, 4.6 × 250 mm, a 15 min gradient of 10–95% aqueous acetonitrile +0.1% TFA, 1 mL/min) to give a mixture of (R)-MTPA esters **2** and **3** (0.3 mg, ratio 5:1) and diester (0.1 mg). **2** (80% of the mixture), t_R = 16.5 min (for

conditions, see above): ^{1}H NMR (CD$_3$OD) δ 7.4–7.7 (5H, ArH), 6.351 (1H, dt, J = 3.0, 1.0 Hz, H-4), 5.08 (1H, m, H-9), 4.79 (1H, brs, H-7), 3.792 (1H, brt, J = 3 Hz, H-5), 3.62 (1H, dd, J = 3.0, 1.5 Hz, H-6), 3.59 (3H, brs, OCH$_3$), 2.17 (1H, m, H-10), 1.65 (1H, m, H-10), 1.45–1.28 (8H, m, H-11–H-14), 0.93 (3H, t, J = 6.5 Hz). **3** (20% of the mixture), t_R = 16.5 min (for conditions, see above): ^{1}H NMR (CD$_3$OD) δ 7.4–7.7 (5H, ArH), 6.21 (1H, brs, H-7), 4.95 (1H, m, H-9), 4.92 (1H, dt, J = 3.0, 1.0 Hz, H-4), 3.72 (1H, dd, J = 3.0, 1.5 Hz, H-6), 3.66 (1H, brt, J = 3 Hz, H-5), 3.63 (3H, brs, OCH$_3$),1.45 (1H, m, H-10), 1.05 (1H, m, H-10), 1.45–1.28 (8H, m, H-11–H-14), 0.91 (3H, t, J = 6.5 Hz). **2 + 3**: LCESIMS (m/z) 507 (M + Na), 485 (M + H).

Integrasone 4- and 7-(S)-MTPA Esters (4 and 5). The (S)-ester mixture (0.4 mg) was prepared, similar to R-esters, by the reaction of (R)-MTPA chloride, affording a ratio of ∼1:2 of **4** and **5**. **4** (33% of the mixture), t_R = 14.0 min (for conditions, see above): ^{1}H NMR (CD$_3$OD) δ 7.4–7.7 (5H, ArH), 6.295 (1H, dt, J = 3.0, 1.0 Hz, H-4), 5.08 (1H, m, H-9), 4.75 (1H, brs, H-7), 3.74 (1H, brt, J = 3 Hz, H-5), 3.65 (3H, brs, OCH$_3$), 3.55 (1H, dd, J = 4.0, 1.5 Hz, H-6), 2.16 (1H, m, H-10), 1.65 (1H, m, H-10), 1.45–1.28 (8H, m, H-11–H-14), 0.91 (3H, t, J = 6.5 Hz). **5** (66% of the mixture), t_R = 14.0 min (for conditions, see above): ^{1}H NMR (CD$_3$OD) δ 7.4–7.7 (5H, ArH), 6.22 (1H, brs, H-7), 5.04 (1H, m, H-9), 4.87 (1H, dt, J = 3.0, 1.0 Hz, H-4), 3.62 (1H, dd, J = 3.5, 2.0 Hz, H-6), 3.61 (1H, brt, J = 3 Hz, H-5), 3.50 (3H, brs, OCH$_3$),1.80 (1H, m, H-10), 1.51 (1H, m, H-10), 1.45–1.28 (8H, m, H-11–H-14), 0.93 (3H, t, J = 6.5 Hz). **4 + 5**: LCESIMS (m/z) 507 (M + Na), 485 (M + H).

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