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Structure and absolute stereochemistry of HIV-1 integrase inhibitor integric acid. A novel eremophilane sesquiterpenoid produced by a *Xylaria* sp.

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Abstract

HIV-1 integrase is critical for viral replication and is absent in the host, and therefore is a potential target for the development of non-toxic antiviral therapy. From the screening of natural product libraries we have discovered integric acid, a novel eremophilane sesquiterpenoid, from a *Xylaria* sp. It inhibited 3′-end processing, strand transfer and disintegration reactions catalyzed by HIV-1 integrase with IC_{50} values of 3–10 μM. The isolation, structure elucidation, relative, and absolute stereochemistry of integric acid are described.

HIV-1 virus is the causative agent of AIDS and remains to be one of the leading causes of human death worldwide. It encodes three enzymes, (i) reverse transcriptase; (ii) protease; and (iii) integrase, that are responsible for viral replication. Of these enzymes, inhibition of reverse transcriptase and protease have lead to important and life saving therapeutic agents. Integrase, the third enzyme, is the only protein that catalyzes the multi-step process of integration that includes cleavage of two bases from the 3′-end of each strand of viral DNA (3′-end processing) and transfer of the processed 3′-ends into the host cell (human) target DNA (strand transfer).[1–4] Integrase is absent in the host and appears to be very specific to HIV-1, and therefore, is a potential target for the development of highly selective anti-HIV antiviral agents.

Screening of libraries of chemicals and natural product extracts are the productive sources for the discovery of inhibitors and ligands of enzymes and receptors, respectively. Using an in vitro assay system and recombinant enzyme,[5] we have recently reported[6,7] the discovery of equisetin and phomasetin from

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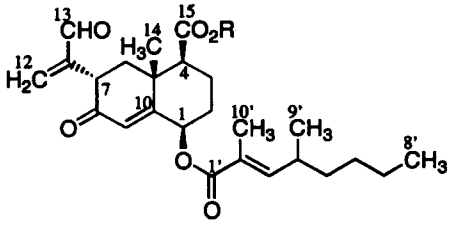


1a: R = H, Integric acid
1b: R = CH_3
1c: R = (*S*)-phenylglycine-OMe
1d: R = (*R*)-phenylglycine-OMe

Figure 1. Structures of integric acid (**1a**), methyl ester (**1b**) and amides (**1c** and **1d**)

the microbial extracts of natural product libraries as inhibitors of HIV-1 integrase. Continued screening of microbial extracts led to the identification of integric acid (**1a**, see Fig. 1) as a unique inhibitor of integrase.

Like equisetin, integric acid inhibited 3′-end processing (IC_{50}=10 μM), and strand transfer (IC_{50}=10 μM) reactions. It also inhibited the disintegration reactions catalyzed by either truncated core domain (52–212 amino acids) or the full-length integrase enzyme with IC_{50} values of 5 and 15 μM, respectively.[7] Most importantly, it inhibited strand transfer reactions catalyzed by pre-integration complexes isolated from HIV-1 infected cells with an IC_{50} of 30 μM.[7] Structurally, integric acid is comprised of an eremophilane sesquiterpenoid which is acylated with 2,4-dimethyl octenoic acid at C-1. In the present communication the isolation, the structure elucidation, relative and absolute stereochemical determination of integric acid are described.

Isolation: Size exclusion chromatography (Sephadex LH-20) of methyl ethyl ketone extracts of the fermentation broth of *Xylaria* sp. (MF6254) followed by reverse phase HPLC on Phenomenex® Primesphere (50×250 mm) column using a linear gradient of 40 to 80% aqueous acetonitrile containing 0.1% trifluoroacetic acid and lyophilization gave 1.0 g of integric acid (1.4 g/L) as a colorless powder, mp. 172–75°C, $[\alpha]_D^{25}$=+38.3 (c, 0.63, CH_3OH).

Structure elucidation: Electron impact (EI) mass spectral analysis of **1a** produced a small molecular ion at *m/z* 430 (4%). High-resolution measurement of the molecular ion (found: 430.2370) suggested a molecular formula $C_{25}H_{34}O_6$ (calcd: 430.2355) that was corroborated by ^1H and ^{13}C NMR spectrum. The molecular formula suggested nine degrees of unsaturation in the molecular structure of integric acid. The UV spectrum of integric acid in CH_3OH showed λ_{max} at 216 (ε=29500) nm. The infra red spectrum of integric acid suggested a band for a carboxyl group (broad 3300–2700) along with multiple carbonyl absorption bands at 1720, 1718, 1690 and 1646 cm^{-1}. The ^1H NMR spectrum (400 MHz) of integric acid in $CDCl_3$ displayed (Table 1) distinct signals for four methyl groups (a methyl singlet at δ 1.50, an allylic methyl doublet at δ 1.80, a methyl doublet at δ 1.00, and a methyl triplet at δ 0.87), an olefinic methine doublet of a quartet at δ 6.54, vinyl group singlets at δ 6.35 and δ 6.10 and an aldehyde group singlet at δ 9.54. The spin systems in the ^1H NMR spectrum were assigned by 2D ^1H–^1H-COSY correlation, which indicated only three contiguous fragments as shown in Fig. 2.

The ^{13}C NMR spectrum (Table 1) of integric acid showed carbon signals for four methyls, two olefinic methines, an olefinic methylene, six methylenes, two aliphatic methines, an oxygenated methine, three olefinic quaternaries, an aliphatic quaternary, an aldehyde, two carboxy type carbonyls and a deshielded carbonyl. The hydrogen bearing carbons in the ^{13}C NMR spectrum were assigned by one-bond correlations using HMQC experiment. An HMBC (J_{CH}=7 Hz) experiment was used for establishing the connectivity between the COSY derived fragments with the remainder of the molecule. Most of the

Table 1
^{1}H and ^{13}C NMR assignment of integric acid (**1a**) and methyl ester (**1b**) in $CDCl_3$

Position	δC	H count	δH, mult, J in Hz	HMBC (H→C)
1	72.65	CH	5.25, t, 2.8	H-9
2	29.97	CH_2	β: 2.15, m	
			α: 1.70, m	
3	20.12	CH_2	β: 1.90, m	H-1, H-2β, H-4
			α: 2.30, m	
4	53.31	CH	2.46, dd, 13.2, 3.2	H-3α, H-14
5	38.28	C°	....	H-1, H-3α, H-4, H-6α, H-9, H-14
6	43.18	CH_2	β: 2.12, dd, 13.2, 4	H-4, H-7, H-14
			α: 2.26, t, 13.6	
7	43.08	CH	3.73, dd, 14.8, 4.4	H-6αβ, H-9, H-12, H-13
8	196.85	C°	....	H-6αβ, H-7
9	129.60	CH	6.10, s	H-1
10	158.90	C°	....	H-1, H-6β, H-14
11	147.66	C°	....	H-7, H-13
12	136.47	CH_2	6.35, s	H-7
			6.25, s	
13	193.13	C°	....	H-12
14	19.53	CH_3	1.50, s	H-6αβ
15	177.87	C°	....	H-4
1'	166.78	C°	....	H-1, H-3', H-10'
2'	125.83	C°	....	H-3', H-4', H-10'
3'	149.69	CH	6.54, dq, 10, 1.2	H-4', H-9', H-10'
4'	33.34	CH	2.50, m	H-3', H-9'
5'	36.53	CH_2	1.3, m	H-3', H-9'
			1.4, m	
6'	22.78	CH_2	1.3, m	H-8'
7'	29.66	CH_2	1.2, m	H-8'
8'	14.08	CH_3	0.87, t, 7.2	....
9'	19.98	CH_3	1.00, d, 6.6	H-3', H-4'
10'	12.65	CH_3	1.80, d, 1.6	H-3'

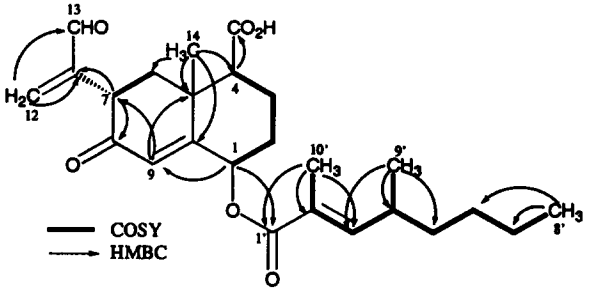


Figure 2. ^{1}H–^{1}H-COSY and selected HMBC correlations of **1a**

two and three bond HMBC correlations are listed in Table 1 and key correlations, which were critical in elucidating the structure of integric acid, are shown in Fig. 2. For example, the correlations of the H-14 (δ 1.50) quaternary methyl group protons to δ 53.31 (C-4), δ 38.28 (C-5), δ 43.18 (C-6) and δ 158.90 (C-10) along with correlations of H-9 to C-5 and C-7; H-1 to C-9; and H-7 to C-8 (δ 193.13) helped in connecting two of the three COSY fragments to each other and completely established the decalin unit. In addition the correlation of H-1 to C-1′ and H-4 to C-15 established the connectivity of the ester linkage at C-1 and the C-15 carboxyl group at C-4, respectively. The HMBC correlation from H-7 to C-11 established the connectivity of C-7 to C-11 and the remaining three-carbon piece containing vinyl aldehyde. The reaction of integric acid with diazomethane produced a mono methyl ester (**1b**, HREIMS: *m/z*: 444.2502). HRMS of integric acid produced two critical fragment ions at *m/z* 260.1078 ($C_{15}H_{16}O_4$)

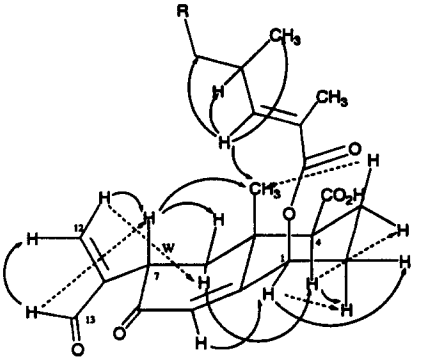


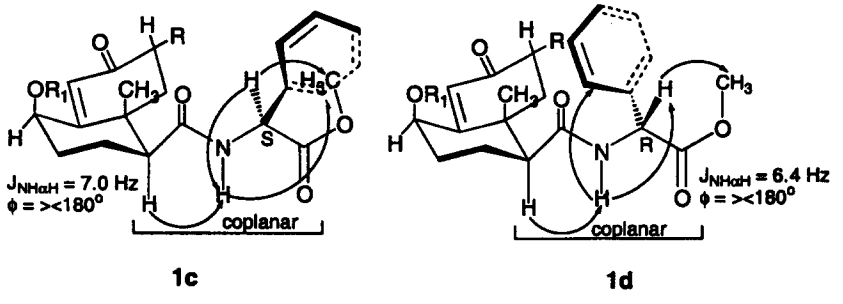
Figure 3. Selected NOESY correlations of **1a** in $CDCl_3$

and *m/z* 153.1284 ($C_{10}H_{17}O$), due to the cleavage of the C-1 oxygen and C-1′ carbonyl bond followed by dehydration, for the decalin unit and the acyl side chain, respectively. Based on these data a flat structure **1a** was assigned to integric acid. Basic hydrolysis of integric acid produced the new 2,4-dimethyloctenoic acid as a gum, $[\alpha]_D^{22}$=+9.5 (c, 0.42, CH_3OH). The stereochemistry of this acid was never assigned.

Relative stereochemistry: The relative stereochemistry of integric acid was deduced by a 2D NOESY experiment at 400 MHz in $CDCl_3$. The correlations are summarized in Fig. 3.

The methyl group at C-14 showed strong NOESY correlations to H-3β, and H-7; H-4 showed strong correlations to H-6α and H-2α establishing the two sets of 1,3-diaxial relationships in β and α face in a quasi-chair–chair conformation of the decalin system. The axial (β) transposition of H-7 was further supported by the NOESY cross peaks between H-12 (δ 6.35) and H-6α (axial, δ 2.26). The correlations of H-9 with H-1 indicated that the H-1 must be equatorially transposed and thus transposing the ester side chain axially at C-1. The stereochemistry at C-4′ could not be reliably established by this experiment.

Absolute stereochemistry: The absolute stereochemistry of integric acid was elucidated by application of the PGME carboxy amide configuration model.[8] Both *S* and *R* phenylglycine methyl ester amides (**1c** and **1d**) were prepared by the reaction of integric acid with respective amino acid hydrochlorides methyl ester using diethylphosphoro cyanidate (DEPC) as a coupling reagent in the presence of triethyl amine in THF. The ^1H NMR spectrum of the two amides **1c** $[\alpha]_D^{22}$=+65.7 (c, 1, CH_3OH), and **1d** $[\alpha]_D^{22}$=–15.3 (c, 0.6, CH_3OH) were thoroughly assigned using 2D COSY. It is imperative for the success of this model that the amide NH must be coplanar with the respective α-proton to the substrate carboxyl group. The protons were indeed coplanar as determined by NOESY measurements as shown in Fig. 4. The differences ($\Delta\delta=\delta S-\delta R$) of the respective chemical shifts of *R*-isomer (**1d**) from *S*-isomer (**1c**) are shown in Fig. 5. These differences indicate *S*-stereochemistry at C-4. Therefore, the absolute stereochemistry of integric acid is 1*R*, 4*S*, 5*R*, and 7*S* and is shown in Fig. 1.



1c **1d**

Figure 4. Selected NOE correlations and conformations of integric acid amides **1c** and **1d** in $CDCl_3$

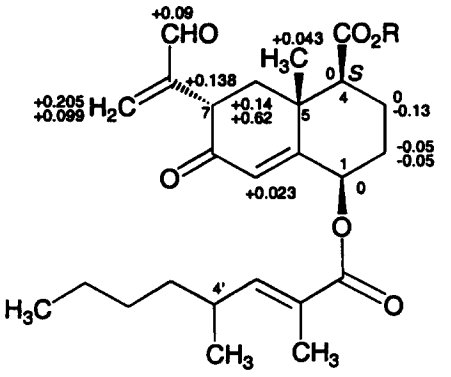


Figure 5. Structure of integric acid showing $\Delta\delta=\delta S-\delta R$ (δ **1c**–δ **1d**)

Integric acid is a member of eremophilane family of sesquiterpenoids. These classes of compounds are produced by both fungus as well as higher plants and display significant variance in the stereochemistry.

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