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Integracides: Tetracyclic Triterpenoid Inhibitors of HIV-1 Integrase Produced by *Fusarium* Sp.

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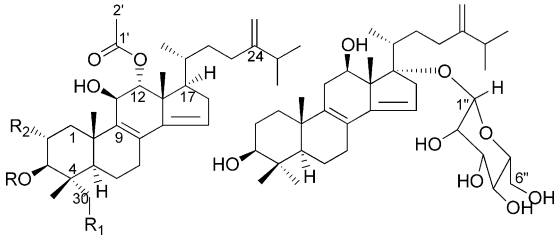
Abstract—HIV-1 integrase is a critical enzyme in the replication of HIV-1. It is absent in the host cells and therefore is a good target for treatment of HIV-1 infections. Integracides are members of the tetracyclic triterpenoids family that were isolated from the fermentation broth of a *Fusarium* sp. Integracide A, a sulfated ester, exhibited significant inhibitory activity against strand transfer reaction of HIV-1 integrase. The discovery, structure elucidation including single crystal X-ray structure and HIV-1 inhibitory activity of these compounds are described.

Introduction

Integration is an essential step in the replication of HIV-1. It is a three step process that includes assembly of proviral DNA onto integrase, endonucleolytic cleavage of the proviral DNA, and strand transfer of the proviral DNA into the host cell DNA.[1] This process is quite distinctive in the retroviral proliferation and the entire reaction is catalyzed by a single viral enzyme called HIV-1 integrase. This enzyme is absent in the mammalian host and therefore presents a safe target for development of anti HIV therapy. Recent discoveries of diketo acid (DKA) based inhibitors helped validate this target for potential anti HIV-1 therapy.[2] No clinically suitable inhibitors of HIV-1 integrase have yet been identified and therefore new classes of inhibitors continue to be important for the success of this program.

Natural products have been very good sources of novel inhibitors for many biological targets. Screening of natural product extracts against recombinant HIV-1 integrase led to the discovery of several classes of natural product inhibitors including equisetin,[3] integric acid,[4] complestatin,[5] integracins,[6] integrastatins[7] and integramycins.[8] Continued screening of fungal extracts led to the discovery of four oxygenated tetracyclic triterpenoids of the 4,4-dimethylergostane family as inhibitors of HIV-1 integrase that include the sulfated ester, integracide A (**1a**), integracide B (**1b**) and two novel derivatives, integracide C (**1c**) and integracide D (**2**). Integracide A (**1a**) inhibited both coupled and strand transfer reactions of recombinant HIV-1 integrase with IC_{50} values of 4 and 9 μM, respectively. The isolation, structure elucidation, stereochemistry by NMR spectroscopy, X-ray crystallographic analysis of a 2-deoxy analogue of **1b** (**1e**) and biological activities of these compounds are herein described.



1a: R = SO_3H, R_1 = H, R_2 = OH
1b: R = R_1 = H, R_2 = OH
1c: R = H, R_1 = R_2 = OH
1d: R = SO_3Na, R_1 = H, R_2 = OH
1e: R = R_1 = R_2 = H

2

Results and Discussion

Producing organism, fermentation and isolation

Filamentous fungus *Fusarium* sp. (ATCC74469), which produced integracides, was isolated from soil collected

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in Tanzania. Initial bioassay guided isolation of **1a** was accomplished from a fermentation broth that was grown on a vermiculite based solid media[9] and was extracted with methyl ethyl ketone and chromatographed on Sephadex LH-20 in MeOH. Fractions eluting in between 0.4 and 0.9 column volumes afforded compound **1a** (1 g/L) as a homogeneous colorless powder. This medium exclusively produced **1a** and therefore isolation was straightforward. However, the vermiculite based medium was not amenable for the large-scale production of **1a** and therefore it was grown in a liquid based AD2 medium.[10] Although the titer of **1a** in this medium was as good as in the solid medium, it also produced **1b** (0.2 g/L) and minor amounts of other compounds including **1c** and **2**. Therefore, a new isolation procedure was developed to isolate these mostly cell bound compounds. The fermentation grown in AD2 medium was filtered and mycelia were extracted with methanol and acetone. Solid phase extraction of the mycelial extracts on SP207 and elution with 55–80% aqueous methanol followed by extraction of aqueous methanolic fractions with ethyl acetate gave **1a** and ~5% of **1b**. Elution of the SP207 column with 80–100% aqueous methanol followed by silica gel chromatography afforded pure **1b**. The fractions eluting with 100% MeOH from the SP207 column produced mixtures of all four compounds that were purified by RPHPLC to give **1a**, **2** (2 mg/L), **1c** (4 mg/L), and **1b**, all as colorless amorphous powders. The SP207 based isolation method provided over 40 g of **1a** of adequate purity that was directly used for the sulfate hydrolysis to provide additional quantities of **1b** in greater than 90% yield.[11]

Integracides A (1a) and B (1b)

ESIMS analysis of **1a** and **1b** gave molecular weights of 594 [m/z 595 $(M+H)^+$, 593 $(M-H)^-$] and 514 [m/z 532 $(M+NH_4)^+$], respectively, revealing molecular formulae $C_{32}H_{50}O_8S$ and $C_{32}H_{50}O_5$, respectively, which were corroborated by ^{13}C NMR spectral analysis. The differences in the molecular formulae of the two compounds indicated that **1a** was a sulfate ester of **1b** and this was confirmed by the corresponding differences in the ^1H and ^{13}C NMR spectrum of the two compounds. Both compounds showed an absorption band at λ_{max} 247 nm in the UV spectrum indicating the presence of a heteroannular diene system. The identities of these compounds were confirmed by spectroscopic and RPHPLC comparisons with respective authentic samples that were previously isolated from another *Fusarium* sp. at Merck as elastase inhibitors.[12] Compound **1a** (A-108835) was re-isolated in 1990 by the Abbott group[13] as an inhibitor of rhinovirus 3C protease. They reported complete spectroscopic data of A-108835, which was identical to the data obtained for **1a**, named herein integracide A. We herein report the full characterization of **1b**. The ^1H and ^{13}C NMR spectrum of **1b** was assigned by COSY, TOCSY, HMQC and HMBC experiments in CD_3OD and $CDCl_3$ and the data in the latter solvent is summarized in Table 1. The chemical shifts of H-3 (δ 2.95, d, $J=10$ Hz in CD_3OD) and C-3 (δ 84.2 in CD_3OD) of **1b** was shifted upfield by Δ 0.93 and Δ 7.7 ppm compared to the corresponding shifts of the sulfate ester **1a**, thus confirming the location of the sulfate ester at C-3. All other shifts of **1a** and **1b** in $CDCl_3$ or CD_3OD (not listed) were within the expected range [$\Delta \pm 2.0$ (^{13}C) and $\Delta \pm 0.1$ (^1H) ppm]. The EIMS of **1b** did not produce a molecular ion but produced three major fragment ions at m/z 454 (M−AcOH], 439 [M−AcOH–CH_3] and 311 (Fig. 1). The protonated form of integracide A (**1a**) tends to decompose upon long term (>months) storage but could be stabilized by conversion to a sodium salt **1d**.

PTSA catalyzed hydrolysis[11] of the sulfate ester of integracide A (**1a**) produced integracide B (**1b**). The structure and stereochemistry of **1b** was further confirmed by a single crystal X-ray crystallographic analysis (Fig. 2) of a 2-deoxy analogue **1e**,[12a] crystallized from acetone–hexane. The ^1H and ^{13}C NMR spectra of **1b** and **1e** was identical except for the obvious differences at C-3 (**1e**: δ_H 3.28, dd, $J=10.5$, 5 Hz, δ_C 78.5 in $CDCl_3$) incurred by the absence of C-2 OH group in the A-ring. The X-ray coordinates have been deposited with the Cambridge Crystallographic Database.[12c] This conclusively established the stereochemistry of this class of compounds as indicated including stereochemistries at C-17 and C-20.

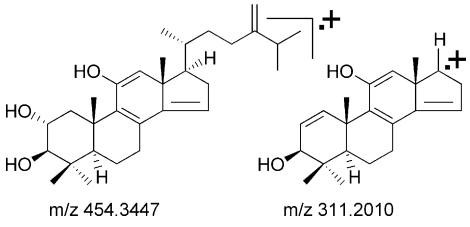

Figure 1. EI–MS fragmentation of **1b**.

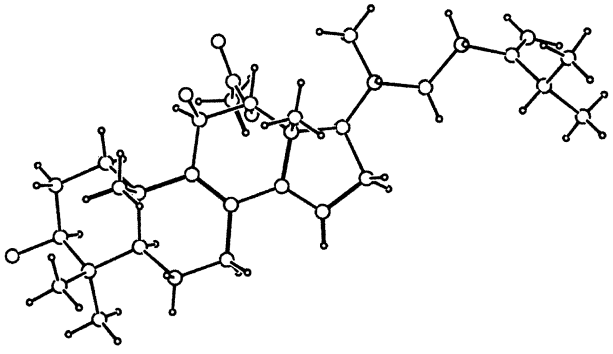

Figure 2. Single crystal X-ray plot of **1e**.

Integracide C (1c)

The EI-MS spectrum of **1c** gave a weak molecular ion at m/z 530 (<5%) and a fragment ion due to a loss of H_2O at m/z 512 which upon high-resolution analysis provided a molecular formula $C_{32}H_{48}O_5$. The ^{13}C NMR spectrum of **1c** (Table 1) afforded signals for 32 carbons including a hydroxymethyl carbon (δ 66.1) at the expense of a methyl group of **1b**. This was consistent with the presence of the extra oxygen in the molecular formula and supported by the corresponding differences

Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) assignments of integracide B (**1b**), integracide C (**1c**), and integracide D (**2**)

	1b in $CDCl_3$		**1c** in CD_3OD			**2** in CD_3OD				
Position	δ_C	δ_H, mult, *J* in Hz	δ_C	δ_H, mult, *J* in Hz	HMBC H→C	NOESY	δ_C	δ_H, mult, *J* in Hz	HMBC H→C	NOESY
1	42.76	eq: 2.37, dd, 12, 4.5 ax: 1.27, t, 12	44.2	eq: 2.34, dd, 12, 4 ax: 1.21, t, 12	C-2, 3, 5, 10	H-11e H-3, 11e	37.4	eq: 1.89, dd, 13, 3.5 ax: 1.35, m	C-3, 5, 10 C-2, 3, 10, 19	H-11e, 19 H-3, 5, 11e
2	69.13	3.81, ddd, 11.5, 10, 4	69.8	3.79, ddd, 11.5, 9.5, 4	C-1, 3	H-19, 29	28.5	1.70, m	C-1, 3, 10	H-3, 19
3	83.40	3.05, d, 9.5	77.8	3.39, d, 9.5	C-1, 4, 29, 30	H-1a, 5	79.3	3.19, dd, 10, 8	C-1, 4, 29, 30	H-1a, 2, 5, 30
4	39.31	—	44.3	—	—		40.1	—	—	
5	50.11	1.27, dd, 12.5, 3	43.5	1.65, m	C-4, 5, 10, 19, 29	H-3	51.8	1.18, dd, 12.5, 2		H-1a, 3
6	18.00	1.77, brdd, 13.5, 7.5 1.69, m	18.9	1.70, m 1.65, m	C-5, 7, 8, 10	H-19, 30	19.4	1.80, m 1.55, m	C-5, 7, 8, 10	H-19
7	26.77	2.45, m 2.32, brdd, 17.5, 7	27.5	eq: 2.46, m ax: 2.28, m	C-6, 8, 9	H-15	28.9	ax: 2.25, m eq: 2.18, m		H-15
8	125.77	—	126.9	—	—		124.1	—	—	
9	138.03	—	139.8	—	—		142.7	—	—	
10	38.37	—	39.3	—	—		38.6	—	—	
11	68.88	4.24, brs	69.5	4.24, t, 0.5	C-8, 9, 12, 13	H-1	31.8	eq: 2.49, ddd, 17, 6, 2 ax: 2.13, dd, 17, 10	C-8, 9, 10, 12, 13 C-8, 9, 10, 12	H-1, 12 H-18, 19
12	79.23	4.97, brd, 2.0	79.9	5.07, dd, 2, 0.5	C-9, 11, 14, 1′	H-18, 21	70.1	3.94, dd, 10.5, 6	C-11, 13, 17, 18	H-11e, 1″, 2″
13	46.58	—	48.0	—	—		58.6	—	—	
14	146.73	—	148.6	—	—		152.2	—	—	
15	121.46	5.61, t, 2.5	121.8	5.45, t, 2	C-8, 13, 14, 16, 17	H-7a, 16	118.2	5.45, t, 2.5	C-8, 13, 14, 16	H-7a, 16
16	35.30	2.45, m 2.06, m	36.3	2.42, ddd, 16, 7.5, 3.5 2.06 m	C-13, 14, 15, 17	H-15, 17	41.2	3.04, dd, 19, 3.5 2.52, d, 19	C-13, 14, 15, 17 C-14, 15, 20	H-15 H-15, 18, 22
17	50.00	1.97, dt, 10.5, 7.5	50.4	1.95, m	C-13, 16, 21	H-16, 21	96.7	—	——	
18	16.74	1.08, s	18.0	1.10, s	C-12, 13, 14, 17	H-12, 20	14.5	0.87, s	C-12, 13, 14, 17	H-11a, 16, 20, 21
19	23.24	1.31, s	24.1	1.34, s	C-2, 5, 9, 10	H-7, 29	20.9	1.08, s	C-2, 5, 9, 10	H-1e, 6, 29
20	33.25	1.65, m	34.5	1.67, m	C-17, 21, 22, 23	H-18, 21	40.6	1.68, m	C-17, 21, 22, 23	H-18
21	18.19	0.88, d, 7	18.7	0.94, d, 6.5	C-17, 20, 22	H-17, 18, 23	15.5	1.17, d, 6.5	C-17, 20, 22	H-18
22	34.45	1.57, m 1.15, m	35.7	1.60, m 1.15, m			32.2	1.73, m 1.62, m		H-16
23	30.90	2.10, m 1.89, m	32.0	2.13, m 1.92, m	C-22, 24, 25, 28	H-21	34.5	2.20, m 1.94, m	C-22, 24, 25, 28	
24	156.58	—	157.5	—			157.9	—		
25	33.79	2.23, hept, 7	34.9	2.25, dhept, 7, 1	C-23, 24, 26, 27, 28		34.8	2.29, dhept, 7, 1	C-23, 24, 26, 27, 28	
26	*21.98	1.03, d, 7	*22.3	1.04, d, 6.5	C-24, 27, 28		*22.3	1.05, d, 7	C-24, 27, 28	
27	*21.85	1.01, d, 7	*22.5	1.03, d, 6.5	C-25, 26, 28		*22.6	1.04, d, 7	C-25, 26, 28	
28	106.09	4.73, brs 4.66, brd, 1.0	107.0	4.74, t, 1.5 4.68, q, 1.5	C-23, 25 C-23, 25		106.8	4.73, brs		
29	16.69	0.89, s	13.7	0.76,s	C-3, 4, 5, 30	H-2, 19	16.2	0.83, s	C-3, 4, 5, 30	H-2, 19
30	28.66	1.06, s	66.1	3.51, d, 11 3.31, d, 11	C-3, 5 C-3	H-3, 6e	28.7	1.02, s	C-3, 4, 5, 29	H-3, 6e
1′	171.19	—	172.1	—			–	—		
2′	21.28	2.05	21.0	1.98, s	C-1′		–	—		
1″							97.7	4.44, d, 0.5	C-17, 2″	H-12, 2″, 3″, 5″
2″							73.2	3.78, d, 3	C-3″, 4″	H-12, 1″, 3″
3″							75.4	3.35, dd, 9.5, 3.5	C-4″	H-1″, 2″, 5″
4″							68.3	3.60, t, 9	C-3″, 5″, 6″	
5″							77.3	3.08, ddd, 9.5, 4.5, 2.5		H-1″, 3″
6″							62.9	3.82, dd, 11.5 3.74, dd, 11.0, 4.5	C-4″, 5″	

in the ^{1}H NMR spectra of both compounds. The ^{1}H and ^{13}C NMR spectrum of **1c** was assigned by 2D NMR (COSY, TOCSY, HMQC, HMBC and NOESY) and is summarized in Table 1. Comparison of the ^{13}C NMR spectum of **1b** and **1c** indicated the absence of the signal for the C-30 methyl group and $\Delta \pm 5$ ppm differences of the chemical shifts of C-3, C-4 and C-5, suggestive of the substitution of the C-30 methyl group with the hydroxymethyl group. This was further supported by 3-bond HMBC correlations of the hydroxymethyl protons (δ 3.51) to C-3 and C-5, and was confirmed by its NOESY (mix time = 300 ms) correlations to H-3 and equatorial H-6 (δ 1.65). The NOESY correlations of H-11 with both H-1 protons, H-12 with H_3-18 and H_3-21 confirmed the β and α stereochemistries at C-11 (OH-axial) and C-12, (OAc-axial), respectively.

Integracide D (2)

The HRESI-FTMS analysis of **2** produced a pseudo molecular ion at m/z 636.4464 ($M + NH_4$) that yielded a molecular formula $C_{36}H_{58}O_8$. The ^{13}C NMR spectrum (Table 1) of **2** corroborated the observed molecular formula and revealed the presence of 36 carbons. The UV spectrum of integracide D was identical to that of the UV spectrum of integracides A–C. The ^{13}C NMR spectrum of **2** showed the absence of signals for the acetyl group, C-2 and C-11 methines compared to **1b** and the presence of signals for an anomeric methine (δ 97.7), four new oxygenated methines and an oxy-methylene (δ 62.9) thus indicating the presence of a glycoside residue. These observations were confirmed by the presence of the corresponding signals in the ^{1}H NMR spectrum of **2**. The ^{1}H and ^{13}C NMR spectra were assigned by respective 2D (COSY, TOCSY, HMQC and HMBC) NMR experiments (Table 1). Analysis of the COSY and the TOCSY spectrum of **2** identified the glycoside as a β- mannopyranoside (H-1″ $J_{vic} = 0.5$ Hz) that was supported by the NOESY correlations of H-1″, H-3″, and H-5″ due to their 1,3-diaxial relationships. H-3 and H-12 appeared as a doublet of doublets at δ 3.19 ($J = 10$, 8 Hz) and δ 3.94 ($J = 10.5$, 6 Hz), respectively, confirming the absences of substitutions at C-2 and C-11. The large J-values (~10 Hz) observed between the vicinal protons suggested the axial orientation of both H-3 and H-12 and was further supported by the NOESY correlations of H-3 with axial H-1, H-5 and H_3-30, and H-12 with equatorial H-11 (δ 2.49). These observations confirmed a β-(equatorial) hydroxy group at C-12, and thus an opposite stereochemistry

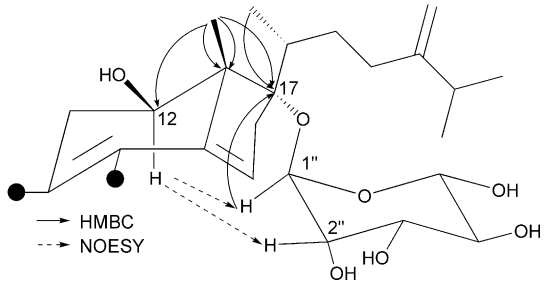

Figure 3. HMBC and NOESY correlations of integracide D (**2**).

Table 2. Biological activities of integracides (**1**–**2**)

Compd	Coupled (IC_{50}, μM)	ST (IC_{50}, μM)	H9 viral spread (CIC_{95}, μM)
1a	4	9	25
1b	82	NT	NT
1c	>100	NT	NT
2	50	NT	NT

compared to the integracides A–C. The HMBC correlation of the anomeric proton H-1″ to δ 96.7 (C-17) established the glycosidic linkage at C-17. The chemical shift of C-17 is unusually downfield shifted, however, the assignment was confirmed by the expected HMBC correlations to H-16, H-18, and H_3-21 (Fig. 3). The H-12 showed NOESY correlations (Fig. 3) to the anomeric proton H-1″ and H-2″ indicating the spatial proximity of these protons at the α-face of the molecule and thus establishing the α-stereochemistry at C-17. Based on these evidences structure **2** was assigned for integracide D.

HIV-1 integrase activity

Integracides were evaluated in coupled and strand transfer assays of recombinant HIV-1 integrase.[2] Integracide A (**1a**) inhibited coupled and strand transfer reactions with IC_{50} (concentration of inhibitors required for 50% inhibition) values of 4 and 9 μM, respectively (Table 2). The desulfated compounds **1b**, **1c** and **2** were significantly less active in the coupled assay and showed IC_{50} values of 82, >100, 50 and >50 μM, respectively. However, these compounds were completely inactive at 100 μM in strand transfer assays and thus indicated the sulfate group was important for the potency.[14] In addition, integracides A inhibited 3′-end processing activity with an IC_{50} value of 5 μM, preintegration complex (PIC) with an IC_{50} value 50 μM and showed antiviral activity with CIC_{95} (concentration of inhibitor required to provide 95% viral protection) = 25 μM in the multiple cycle H9 viral spread assay.[2a] Unfortunately, it also showed toxicity at 25 μM and did not exhibit any therapeutic window.

In summary, we have described four triterpenoid natural products of fungal origin belonging to the 4,4-dimethylergostane family that displayed a range of HIV-1 integrase inhibitory activities.

Experimental

For general experimental procedure, see ref 9a.

Fermentation of *Fusarium* sp (ATCC 74469)

The culture was grown 2 days at 25 °C in a seed medium, which has been described earlier.[9] A 12 mL inoculum was transferred to the production medium (PBGG1)[9] prepared by transferring 200 mL liquid to 675 cc large particle vermiculite in a 2-L roller jar. The roller jars were incubated at 22 °C for 18 days. In an alternate experiment, the production medium was a liquid based AD2 medium.[10] A 50 mL aliquot of the liquid was

transferred to 250 mL Erlenmeyer flasks. Aliquots of 1–2 mL of the seed was used for inoculation of these flasks which were incubated at 22 °C for 7–21 days.

Initial isolation of integracide A (1a)

The *Fusarium* sp. was grown on vermiculite and was extracted with 1.2 volume methyl ethyl ketone (MEK) by shaking at a shaker for 30–60 min. Sixty millilitres of the MEK extract was concentrated to dryness and the residual water was removed by lyophilization to give 180 mg of pale residue. This material was dissolved in 5 mL methanol–methylene chloride (1:1) and was charged on to a 1L Sephadex LH-20 column packed in methanol. Twenty millilitres of each fractions were collected at a flow rate of ~20 mL/min. The activity eluted from 400 to 900 mL of the elution volume of methanol. The combined active fractions were concentrated to give 64.4 mg of homogeneous integracide A (**1a**) as a colorless powder, $t_R = 12.5$ min (Zorbax RX C-8, 4.6×250 mm, 60% aqueous CH_3CN +0.1% TFA, 1 mL/min), $[\alpha]_D^{23} = +13.7°$ (*c* 7, MeOH), UV λ_{max} (MeOH): 247 ($\varepsilon = 14{,}650$), ^1H NMR (acetone-d_6 + 10% CD_3OD) δ 5.56 (1H, brs, H-15), 5.04 (1H, brd, $J = 1.2$ Hz, H-12), 4.70, 4.66 (1H each, brs, H-28), 4.24 (1H, brs, H-11), 3.91 (1H, brdt, $J = 10$, 4 Hz, H-2), 3.82 (1H, d, $J = 10$ Hz, H-3), 2.44 (1H, m, H-16), 2.38 (1H, dd, 12, 4 Hz, H-1β), 2.37 (1H, m, H-7α), 2.30 (1H, m, H-7β), 2.23 (1H, hept, $J = 6.8$ Hz, H-25), 2.14 (1H, m, H-23), 2.05 (1H, m, H-16), 2.00 (1H, m, H-17), 1.95 (3H, s, H_3-32), 1.91 (1H, m, H-23), 1.77 (1H, m, H-6), 1.69 (1H, m, H-6), 1.68 (1H, m, H-20), 1.58 (1H, m, H-22), 1.31 (3H, s, H_3-19), 1.27 (1H, m, H-1α), 1.23 (1H, m, H-5), 1.14 (1H, m, H-22), 1.09 (3H, s, H_3-18), 1.08 (3H, s, H_3-30), 1.01, 1.00 (6H, d, $J = 6.8$ Hz, H_3-26, H_3-27), 0.91 (3H, d, $J = 6.4$ Hz, H_3-21), 0.87 (3H, s, H_3-29); ^{13}C NMR (acetone-d_6 + 10% CD_3OD) δ 170.70 (C-31), 157.12 (C-24), 148.37 (C-14), 140.00 (C-9), 125.47 (C-8), 120.98 (C-15), 106.71 (C-28), 90.11 (C-3), 78.99 (C-12), 69.14 (C-11), 68.20 (C-2), 51.39 (C-5), 49.80 (C-17), 47.68 (C-13), 44.15 (C-1), 40.49 (C-4), 38.56 (C-10), 35.95 (C-16), 35.30 (C-22), 34.35 (C-25), 34.14 (C-20), 31.62 (C-23), 29.22 (C-30), 27.39 (C-7), 23.37 (C-19), 22.30, 22.15 (C-26, C-27), 21.24 (C-32), 18.92 (C-6), 18.50 (C-21), 17.96 (C-29), 17.10 (C-18); ESI-MS: *m/z* 595 $[M+H]^+$, 593 $[M-H]^-$; EI–MS: *m/z* 436 $[M-H_2SO_4-AcOH]^+$.

Large scale isolation of integracides A (1a), B (1b), C (1c) and D (2)

A 9 L fermentation broth (pH 7.0) grown for 19 days on a liquid AD2 media[10] was filtered through Celite. The filtrate contained small amounts of integracides A (**1a**) and B (**1b**) and was discarded. The mycelia was extracted twice each with 4 L methanol followed by 8 L of acetone. The combined acetone extract was concentrated to almost dryness and then combined with the methanol extract. The combined extracts were diluted with 8 L of water and charged over a 2 L SP207 column at a flow rate of 100 mL/min. The column was thoroughly washed with 50% aqueous methanol until the eluent became almost colorless. Elution with 70% aqueous methanol (16 L) gave fraction A, which contained almost exclusively **1a** (11 g). Subsequent elution with 100% methanol (6 L) and acetone (4 L) gave fraction B that possessed a mixture of **1a** (3.5 g) and **1b** (4.8 g). The production yield was estimated by analytical HPLC. The fraction B also contained minor amounts of related congeners. An aliquot (2.8 g) of fraction B was dissolved in 8 mL methanol and 1 mL each was chromatographed, in eight equal runs, on a reverse phase HPLC (Zorbax RX C-8, 22×250 mm, 40 min gradient of 30–70% aq CH_3CN at a flow rate of 8 mL/min). Lyophilization of fractions eluting between 24 and 28 min gave **1a** (1.04 g) and lyophilization of fractions eluting between 65 and 77 min gave **1b** (0.26 g) both as colorless powders. Lyophilization of fractions eluting at 39 and 44–48 min gave 25 and 40 mg each of integracides D (**2**) and C (**1c**), respectively as colorless powder.

Integracide B (1b). Colorless powder, $t_R = 18.1$ min (70% aq CH_3CN +0.1% TFA, 1 mL/min), $[\alpha]_D^{23} = +7.9°$ (*c* 5, MeOH), UV λ_{max} (MeOH): 247 ($\varepsilon = 15220$), ^1H and ^{13}C NMR (see Table 1), ESI-MS (*m/z*): 1046 $[2M+NH_4]^+$, 532 $[M+NH_4]^+$, 497 $[M+H-H_2O]^+$, 437 $[M+H-H_2O\text{–}HOAc]^+$, 1141 $[2M-TFA-H]^-$, 627 $[M-TFA-H]^-$. HR-EI–MS (*m/z*): 454.3447 (M−HOAc, calcd for $C_{30}H_{46}O_3$: 454.3448), 311.2010 (calcd for $C_{21}H_{27}O_2$: 454.3448).

Integracide C (1c). Colorless powder, $t_R = 14.8$ min (70% aq CH_3CN +0.1% TFA, 1 mL/min), $[\alpha]_D^{23} = +10.3°$ (*c* 1.65, MeOH), UV λ_{max} (MeOH): 248 ($\varepsilon = 14{,}110$), ^1H and ^{13}C NMR (see Table 1), EI–MS (*m/z*): 530 [5%, M]$^+$, HR-EI–MS (*m/z*): 512.3493 (M−H_2O, calcd for $C_{32}H_{48}O_5$: 512.3503), 470.3393 (M−HOAc, calcd for $C_{30}H_{46}O_4$: 470.3398), 455.3169 (calcd for $C_{29}H_{43}O_4$: 455.3163), 331.1925 (calcd for $C_{20}H_{27}O_4$: 331.1910), 159.0815 (calcd for $C_{11}H_{11}O$: 159.0810).

Integracide D (2). Colorless powder, $t_R = 8.4$ min (70% aqueous CH_3CN +0.1% TFA, 1 mL/min), $[\alpha]_D^{23} = -15.2°$ (*c*, 2.8, MeOH), UV λ_{max} (MeOH): 247 ($\varepsilon = 15{,}160$), ^1H and ^{13}C NMR (see Table 1), ESI-MS (*m/z*): 1872 $[3M+NH_4]^+$, 1254 $[2M+NH_4]^+$, 636 $[M+NH_4]^+$, 601 $[M+H\text{–}H_2O]^+$, 1349 $[M+TFA-H]^-$, 731 $[M+TFA-H]^-$. HR-ESI-MS (*m/z*): 636.4464 (calcd for $C_{36}H_{58}O_8 + NH_4$: 636.4477).

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