Isolation, Structure, and HIV-1 Integrase Inhibitory Activity of Xanthoviridicatin E and F, Two Novel Fungal Metabolites Produced by *Penicillium chrysogenum*

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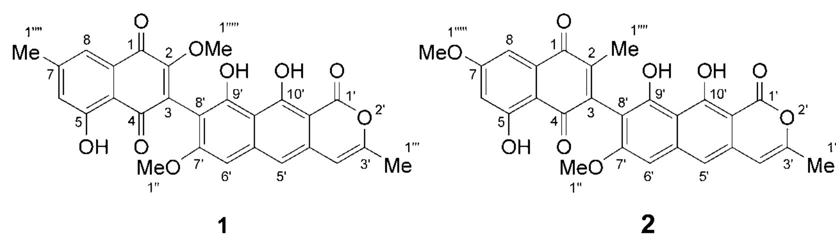
HIV-1 Integrase is a critical enzyme for replication of HIV, and its inhibition is one of the most promising new drug targets for anti-retroviral therapy with potentially significant advantages over existing therapies. Xanthoviridicatins E (**1**) and F (**2**) are two novel polyketide natural products that were isolated from a fermentation broth of an endophytic strain of *Penicillium chrysogenum* isolated from the living leaves collected in Peru. These compounds are new members of the unsymmetrical xanthoviridicatin family represented by the broader xanthomegnin family. Xanthoviridicatins E and F inhibited the cleavage reaction of HIV-1 integrase with an IC_{50} of 6 and 5 μM, respectively. The bioassay-directed isolation, structure elucidation, and HIV-1 inhibitory activity of these compounds are described.

1. Introduction. – HIV-1 Integrase is one of 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 (for recent reviews on HIV integrase, see [1]). The other two key enzymes are reverse transcriptase and protease. The inhibitors of the latter two enzymes have led to many clinical agents that continue to have enormous impact on the control of spread of HIV-1 infections. However, the emergence of multi-drug resistant virus, 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. HIV-1 Integrase is absent in the host cells and is required for viral replication. The inhibition of integration is one of the most suitable targets that could lead to a completely new mode of action and plausibly nontoxic agents. Recently, much progress has been made in identification of inhibitors of this enzyme [2].

Natural products have been very good sources of novel inhibitors for many biological targets, most importantly of anti-infective targets. Screening of natural-product extracts against recombinant HIV-1 integrase led to the discovery of several classes of natural-product inhibitors exemplified by equisetin [3], complestatins [4], and chaetochromins [5]. Continued screening of a collection of fungal extracts led to the discovery of two novel polyketide-derived natural products named herein xanthoviridicatins E (**1**) and F (**2**) produced by *Penicillium chrysogenum* THOM. The bioassay-directed isolation, structure elucidation, and HIV-1 inhibitory activity of these compounds are described.



2. Results and Discussion. – *Isolation*. The producing fungus was isolated from the living leaves of an unidentified plant collected in Camino Cuzco Amazonico in Peru, following the method described by *Pelaez* and co-workers [6]. The culture was identified as *Penicillium chrysogenum* based on a combination of micromorphological and cultural characteristics [7], as described in the *Exper. Part*, and is preserved in the *Merck* Microbial Collection with the culture accession number MF 6389. The culture was grown in CYS80 medium [8] as detailed in the *Exper. Part*. The culture broth was extracted with methyl ethyl ketone and was first chromatographed on a column of *Sephadex LH-20* with MeOH. The fractions active in a HIV-1 integrase coupled assay were chromatographed on reversed-phase HPLC to afford xanthoviridicatins E (**1**; 114 mg/l) and F (**2**; 48 mg/l) as yellow powders.

Xanthoviridicatin E (**1**). The high-resolution MS analysis of **1** by ESI and EI methods afforded a molecular formula of $C_{27}H_{20}O_9$ (*m/z* 488.1099), indicating the presence of 18 degrees of unsaturation. The structure **1** was assigned to xanthoviridicatin E by the analysis of the ^{1}H- and ^{13}C-NMR, HMQC, and HMBC spectra.

The EI-MS of **1** did not show significant fragmentation except for the loss of H_2O and MeOH. The UV spectrum showed absorption maxima at 234, 284, and 392 nm. The IR spectrum displayed absorption bands for conjugated ketone (1629 cm^{-1}), lactone (1689 cm^{-1}), and OH groups (3389 cm^{-1}). The ^{13}C-NMR spectrum revealed the presence of signals for 27 C-atoms and confirmed the molecular formula. Of those, nine C-atoms were proton-bearing and the remaining 18 were of sp^2 and conjugated-ketone types. The ^{1}H-NMR spectrum ($CDCl_3$) displayed two olefinic Me *s* at δ 2.28 and 2.43, two aromatic MeO *s* at δ 3.87 and 3.91, three olefinic/aromatic proton *s* at δ 6.26, 6.70, and 7.02, an aromatic Me *d* at δ 7.50 ($J = 1.6$ Hz), and proton *m* at δ 7.06, and three exchangeable phenolic protons at δ 9.71, 12.22, and 13.62 as reasonably sharp *s*. The proton-bearing C-atoms were assigned by an HMQC experiment, and the assignments are listed in the *Table*. Information revealed by these experiments provided a general framework of the structure and allowed the elucidation of the full structure by an HMBC experiment recorded at ^nJ(XH) = 7 Hz in $CDCl_3$. The HMBC correlations of CH_3(1''') to C(3') (δ 153.2) and C(4') (δ 104.8), of *H*–C(4') to CH_3(1''') (δ 19.6), C(3'), C(5') (δ 112.7), and highly shielded C(10'a) (δ 98.4; assigned *ortho* to the two O-bearing C(1') and C(10')); of H–C(5') to C(10'a), C(9'a) (δ 107.9), C(5'a) (δ 141.6), and C(4'), and of *H*–C(6') to C(9'a), C(8') (δ 105.8), C(7') (δ 160.8), and C(5') established the tricyclic system (see *Fig. 1*). The phenolic protons at δ 13.62 and 9.71 gave strong HMBC correlations to C(10'a), C(10') (δ 162.3), and C(9'a) and to C(9'a), C(9') (δ 155.4), and C(8'), respectively, confirming their positions at C(10') and C(9') respectively. The CH_3O protons (δ 3.87) produced an HMBC correlation to C(7') (δ 160.8) establishing its location at C(7'). These correlations fully established the tricyclic system of xanthoviridicatin E. The remaining bicyclic part of xanthoviridicatin E was accordingly elucidated by the HMBC correlations of *H*–C(6) to C(4a) (δ 112.8), C(8) (δ 120.6), and C(9) (δ 22.2) and of *H*–C(8) to C(1) (δ 181.2), C(4a), C(6) (δ 124.6), and also to C(9), thus establishing the OH and Me substitutions at C(5) and C(7), respectively. This substitution pattern was further corroborated by the HMBC correlations of CH_3(1'''') to C(6), C(7), and C(8), and of O*H*–C(5) (δ 12.22) to C(4a), C(5), and C(6).

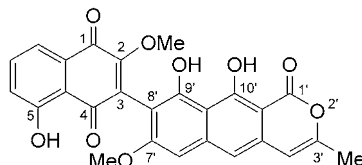
The ^{13}C-NMR shifts of the quaternary C-atoms that did not exhibit any HMBC correlations were assigned based on the substitution-induced chemical-shift values of aromatic C-atoms. The δ(C) values of **1** were

Table. *¹H-*(400 MHz) *and ¹³C-NMR*(100 MHz) *Assignments of* **1** *and* **2** *in $CDCl_3$*[1]). δ in ppm, *J* in Hz.

	1		**2**	
	δ(C)	δ(H)	δ(C)	δ(H)
C(1′)	168.3		168.3	
C(10′a)	98.4		98.4	
C(10′)	162.3		162.3	
C(9′a)	107.9		108.1	
C(9′)	155.4		154.8	
C(8′)	105.8		107.9	
C(7′)	160.8		160.3	
H−C(6′)	97.8	6.70 (*s*)	97.9	6.71 (*s*)
C(5′a)	141.6		141.6	
H−C(5′)	112.7	7.02 (*s*)	112.7	7.04 (*s*)
C(4′a)	132.5		132.5	
H−C(4′)	104.8	6.26 (*s*)	104.8	6.27 (*s*)
C(3′)	153.2		153.2	
Me(1‴)	19.6	2.28 (*s*)	19.3	2.29 (*s*)
Me(1″)O	56.2	3.87 (*s*)	56.1	3.86 (*s*)
C(1)	181.2		184.7	
C(2)	159.1		147.8[a])	
C(3)	123.2		140.8[a])	
C(4)	189.5		187.2	
C(4a)	112.8		110.0	
C(5)	161.5		164.3	
H−C(6)	124.6	7.06 (*m*)	106.3	6.65 (*d*, *J* = 2.8)
C(7)	147.1		165.8	
H−C(8)	120.6	7.50 (*d*, *J* = 1.6)	107.5	7.23 (*d*, *J* = 2.8)
C(8a)	131.6		134.1	
Me(1⁗′)	22.2	2.43 (br. *s*)	14.6	2.02 (*s*)
Me(1⁗″)O	60.5	3.91 (*s*)	56.2	3.90 (*s*)
OH−C(10′)		13.62 (*s*)		13.63 (*s*)
OH−C(9′)		9.71 (br. *s*)		9.66 (br. *s*)
OH−C(5)		12.22 (*s*)		12.35 (*s*)

[a]) Assignments may be reversed.

consistent with the corresponding δ(C) of xanthoviridicatin D (**3**) [9]. However, the published δ(C) values [9] for C(10′a) and C(8′), and for C(10′) and C(9′) of **3** must be reversed.



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Xanthoviridicatin F (**2**). The HR-ESI-FT-MS analysis of **2** afforded a molecular formula $C_{27}H_{20}O_9$, *i.e.*, **2** is isomeric to **1**, which was supported by essentially similar UV, IR, ¹H-, and ¹³C-NMR spectra (*Table*). While the ¹H- and ¹³C-NMR spectra of the tricyclic moieties of **1** and **2** were identical, the spectra of the bicyclic moieties were

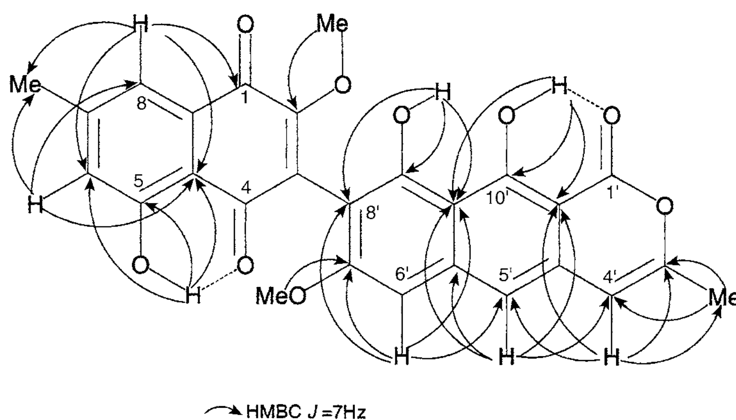

Fig. 1. *HMBC Correlations of xanthoviridicatin E* (**1**)

different. The NMR spectral data of **2** indicated that C(2), C(5), and C(7) bear substituents just like **1**, but the position of the Me and the MeO groups must be reversed. Similarly to the structure of **1**, the structure of **2** was confirmed by the HMBC correlations.

As expected, the HMBC correlations exhibited by the tricyclic moiety of **2** were identical to the corresponding HMBC correlations of **1**, thus confirming the similarity of the tricyclic parts of the two congeners (see *Fig. 2*). Both H–C(6) and H–C(8) of **2** experienced upfield shifts by $\Delta\delta(\mathrm{H}) = -0.41$ and -0.27 ppm, respectively. The $\delta(\mathrm{C})$ of C(6) and C(8) were also upfield shifted by $\Delta\delta = -18.3$ and -13.1 ppm, respectively. These upfield shifts indicated that C(7) must have an O-substitution, *i.e.*, C(7) bears a MeO group. This was confirmed by the HMBC correlation of CH_3O (δ 3.90) to C(7) (δ 165.8). The $\delta(\mathrm{H})$ and $\delta(\mathrm{C})$ of the Me group also experienced upfield shifts by $\Delta\delta(\mathrm{H}) = -0.41$ and $\Delta\delta(\mathrm{C}) = -7.6$ ppm, respectively. $CH_3(1'''')$ (δ 2.02) produced strong HMBC correlations to C(1) (δ 184.7), C(2) (δ 147.8), and C(3) (δ 140.8) confirming its position at C(2) and the structure of xanthoviridicatin F (**2**).

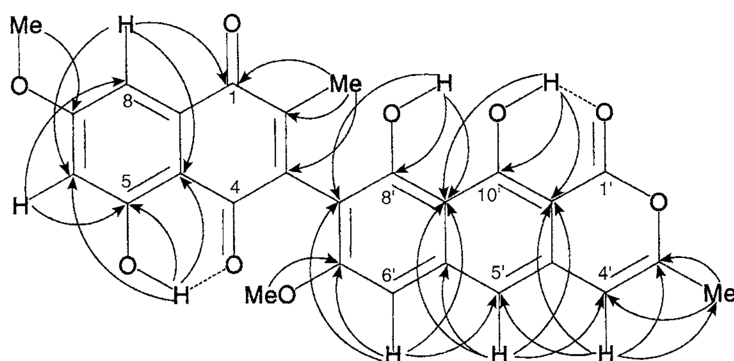

Fig. 2. *HMBC Correlations of xanthoviridicatin F* (**2**)

HIV-1 Integrase Activity. Xanthoviridicatins E (**1**) and F (**2**) were evaluated for their ability to inhibit HIV-1 integrase assays, including coupled and strand-transfer assays. The details of the assays have been described in [2][3b]. Compounds **1** and **2** inhibited

the HIV-1 integrase activity with IC_{50} values of 6 and 5 μM, respectively, as measured in coupled assay, and did not inhibit the strand-transfer reaction ($IC_{50} > 100$ μM). This indicated that these compounds are mostly cleavage inhibitors.

In summary, two novel polyketides of the xanthoviridicatin family are described that are inhibitors of the cleavage reaction of HIV-1 integrase. Xanthoviridicatin E (**1**) is the 3′,4′-didehydro-methyl analog of xanthoviridicatin D (**3**). The reversal of the Me and the MeO substituents at C(7) and C(2) of **1** and **2** suggests an interesting biogenesis of the two polyketide congeners.

Experimental Part

General. For general experimental details, see [10]. UV Spectra: λ_{max} (log ε) in nm. IR Spectra: $\tilde{\nu}$ in cm^{-1}. MS: in *m/z* (rel. %).

HIV-1 Integrase Assay. For the HIV-1 integrase assays, see [2][3b].

Isolation and Identification of the Producing Organism. The producing organism MF 6389, an endophyte that was isolated from living leaves of an unidentified plant collected in Camino Cuzco Amazonico, Peru, following the method of surface sterilization by serial washing in EtOH/*Chlorox*/EtOH [6]. The fungus was identified as *Penicillium chrysogenum* based on a combination of characteristics, including penicillate conidiophores typically terverticillate, smooth walled, mostly with two rami, bearing subspheroidal to ellipsoidal conidia, which are smooth walled *ca*. 3 μm long when mature. The colonies developed rapidly on the standard media used for the identification of *Penicillium* species [7]. On *Czapek* yeast-extract agar (CYA), they attained 40 mm after 7 days of incubation at 25° and produced blue-green conidia in mass and a yellow soluble pigment. On malt-extract agar (MEA), colonies attained 28 mm at 25°, and were velutinose, low, and sparse. On 25% glycerol nitrate agar (G25N), the colonies attained 22 mm at 25°, and were radially sulcate and dense; at 5°, the formation of a microcolony was observed, and at 37°, there was no growth.

Fermentation of Penicillium chrysogenum. For the production of the compound, seed flasks were prepared from potato-dextrose agar (PDA, *Difco*) by using fresh slants of the isolate MF 6389 as described [8]. Two-ml portions of the resulting cultures were used to inoculate 250-ml unbaffled *Erlenmeyer* flasks containing 50 ml of CYS80 medium, which were incubated at 25° in a rotatory shaker at 220 rpm for 21 days. The CYS80 medium contains (in g/l): *c*orn meal (50), *y*east extract (1), and *s*ucrose (80). For detailed descriptions of the medium and growth conditions, see [8].

Extraction and Isolation. The fermentation broth (250 ml) was extracted with 300 ml of methyl ethyl ketone by shaking for 30 min. The methyl ethyl ketone layer was separated, and a 60-ml aliquot was evaporated to give 350 mg of dark brown solid, which was dissolved in a MeOH/CH_2Cl_2 1:1 (30 ml) and chromatographed (1-l column, *Sephadex LH-20*; MeOH, flow rate 5 ml/min; 4-min (20-ml) fractions). All fractions were evaluated in the HIV-1 integrase assay. The activity was highly retained and eluted in 1.12 – 1.6 column volumes. The active fractions were pooled and evaporated to afford 37.4 mg of yellow powder that was submitted to reversed-phase HPLC (*Zorbax RX C-8*, 21 × 250 nm column; 60-min gradient of 20 → 80% aq. MeCN + 0.1% CF_3COOH, flow rate 8 ml/min). The activity was highly retained and eluted in between 71 – 73 and 75 – 77 min. These two sets of fractions were pooled, concentrated under reduced pressure, and lyophilized: **1** (6.8 mg) and **2** (2.9 mg), in order of elution, as yellow powders.

Xanthoviridicatin E (*= 3-(9,10-Dihydroxy-3-methyl-1-oxo-1*H*-naphtho[2,3-*c*]pyran-8-yl)-5-hydroxy-2-methoxy-7-methylnaphthalene-1,4-dione*; **1**): t_R 6.35 min (*Zorbax RX C-8*, 4.6 × 250 nm, 80% aq. MeCN + 0.1% CF_3COOH, 1 ml/min. UV ($CHCl_3$): 392 (3.89), 300 (sh), 284 (4.74), 272 (sh), 234 (4.54). IR (ZnSe): 3389, 3006, 1679, 1629, 1457, 1414, 1371, 1316, 1299, 1276, 1242, 1160, 1088, 1025. ^{1}H- and ^{13}C-NMR: *Table*. HR-ESI-FT-MS: 489.1197 ($[M+H]^+$, $C_{27}H_{21}O_9^+$; calc. 489.1180). HR-EI-MS: 488.1099 (42, M^+, $C_{27}H_{20}O_9^+$; calc. 488.1107), 456.0837 (100, $[M-\text{MeOH}]^+$, $C_{26}H_{16}O_8^+$; calc. 456.0845), 385.0659 (21, $[M-2\times\text{MeOH}-2\times H_2O]^+$, $C_{23}H_{13}O_6^+$; calc. 385.0712).

Xanthoviridicatin F (*= 3-(9,10-Dihydroxy-3-methyl-1-oxo-1*H*-naphtho[2,3-*c*]pyran-8-yl)-5-hydroxy-7-methoxy-2-methylnaphthalene-1,4-dione*; **2**): t_R 7.31 min (*Zorbax RX C-8*, 4.6 × 250 nm, 80% aq. MeCN + 0.1% CF_3COOH, 1 ml/min). UV ($CHCl_3$): 390 (3.76), 300 (sh), 282 (4.61), 272 (4.52), 234 (4.51). IR (ZnSe): 3387, 2925, 2854, 1679, 1630, 1414, 1386, 1315, 1272, 1245, 1208, 1160, 1089, 1052, 976. ^{1}H- and ^{13}C-NMR: *Table*. HR-ESI-FT-MS: 489.1206 ($[M+H]^+$, $C_{27}H_{21}O_9^+$; calc. 489.1180). HR-EI-MS: 488.1107 (100, M^+, $C_{27}H_{20}O_9^+$;

calc. 488.1107), 473.0918 (53, $[M - Me]^+$, $C_{26}H_{17}O_9^+$; calc. 473.0873), 458.0683 (13, $[M - 2 \times Me]$, $C_{25}H_{14}O_9^+$; calc. 458.0638), 385.0692 (5, $[M - 2 \times MeOH - 2 \times H_2O]^+$, $C_{23}H_{13}O_6^+$; calc. 385.0712).

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