ORIGINAL ARTICLE

Xanthoradones, new potentiators of imipenem activity against methicillin-resistant *Staphylococcus aureus*, produced by *Penicillium radicum* FKI-3765-2 II. Structure elucidation

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The structures of xanthoradones A and B, new potentiators of imipenem activity against methicillin-resistant *Staphylococcus aureus* produced by *Penicillium radicum* FKI-3765-2, were elucidated by spectroscopic studies, including various NMR experiments. These compounds have an asymmetric biaryl skeleton, which contains dihydronaphthopyranone and naphthoquinone moieties.

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INTRODUCTION

Two new compounds, designated xanthoradones A and B (Figure 1), were isolated as potentiators of imipenem activity against methicillin-resistant *Staphylococcus aureus* from the culture broth of *Penicillium radicum* strain FKI-3765-2.¹ The taxonomy of the producing strain, fermentation, isolation and biological properties of the xanthoradones were described in a previous paper.¹ In this study, the physico-chemical properties and structure elucidation of xanthoradones are described.

RESULTS

Physico-chemical properties

The physico-chemical properties of xanthoradones A and B are summarized in Table 1. They had similar UV spectra with absorption maxima at 218 nm, 263–267 nm and 373–375 nm. The IR absorption at 1606–1778 cm⁻¹ and 3403–3407 cm⁻¹ suggested the presence of carbonyl and hydroxyl groups in their structures. These data indicated that they share the same skeleton.

Structure elucidation of xanthoradone A

The molecular formula of xanthoradone A was determined to be C₂₇H₂₂O₉ on the basis of high-resolution electrospray ionization time-of-flight mass spectrum (HRESI-TOF-MS) measurement. The ¹³C NMR spectrum (in CDCl₃) showed 27 resolved signals, which were classified into two methyl carbons, one methylene carbon, four sp² methine carbons, two oxygenated methyl carbons, one oxygenated methine carbon, nine sp² quaternary carbons, five oxygenated sp²

quaternary carbons and three carbonyl carbons by analyzing the DEPT and heteronuclear single quantum coherence (HSQC) spectra. The ¹H NMR spectrum (in CDCl₃) displayed 22 proton signals, nine of which were suggested to be three hydroxy protons (δ 9.73, 12.5 and 13.9) and two oxygenated methyl protons (δ 3.84 and 3.92), as reported for xanthoviridicatin F.² These results supported the molecular formula. The connectivity of proton and carbon atoms was established by the ¹³C–¹H HSQC spectrum (Table 2). Analyses of ¹H–¹H COSY revealed the presence of the partial structure I, as shown in Figure 2a. Furthermore, ¹³C–¹H long-range couplings of ²J and ³J observed in the ¹³C–¹H heteronuclear multiple bond coherence (HMBC) spectrum gave the following linkages (Figure 2b): (1) Cross peaks from H₂-3 (δ 3.00, 3.06) to C-4 (δ 133.5), C-5 (δ 116.1) and C-13 (δ 99.7); from H-5 (δ 6.99) to C-3 (δ 34.7), C-6 (δ 140.0), C-7 (δ 98.1), C-11 (δ 108.4) and C-13; from H-7 (δ 6.71) to C-5, C-8 (δ 160.3), C-9 (δ 109.0) and C-11; from OH-10 (δ 9.73) to C-9, C-10 (δ 154.7) and C-11; from OH-12 (δ 13.9) to C-11, C-12 (δ 162.7) and C-13; and from H₃-15 (δ 3.84) to C-8 indicated that a naphthalene skeleton connects the partial structure I at C-4. Furthermore, the findings that the chemical shift of C-2 (δ 76.8) corresponds to that of an oxygenated carbon and that the OH-12 proton (δ 13.9) shifted to a lower field because of hydrogen bonding indicated that C-2 and C-13 are connected through an ester bond. This connection was supported by the chemical shift of C-1 (δ 171.5) corresponding to an ester bond and the long-range coupling of ⁴J observed from H-5 to C-1 in ¹³C–¹H HMBC (Figure 2). Thus, these data indicated that xanthoradone A was found to have a dihydronaphthopyranone skeleton. (2)

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Figure 1 Structures of xanthoradones A, B and xanthoviridicatin D to F.

Table 1 Physico-chemical properties of xanthoradones A and B

	Xanthoradone A	Xanthoradone B
Appearance	Orange crystal	Orange crystal
Molecular weight	490	490
Molecular formula	C ₂₇ H ₂₂ O ₉	C ₂₇ H ₂₂ O ₉
HRESI-TOF-MS (<i>m/z</i>)		
Calculated	489.1186 (M-H) ⁻	489.1186 (M-H) ⁻
Found	489.1136 (M-H) ⁻	489.1149 (M-H) ⁻
UV (MeOH), λ _{max}	218 (32 600), 263	218 (55 300), 267
nm (ε)	(53 400), 375 (15 100)	(34 700), 373 (13 000)
[α] _D ²⁶	+320.1° (c=0.1, CHCl ₃)	+167.9° (c=0.1, CHCl ₃)
IR (KBr), ν _{max} (cm ⁻¹)	3407, 2971, 1778, 1681, 1631, 1598	3403, 2967, 1641, 1606, 1579, 1542

Cross peaks from H-2' (δ 6.08) to C-1' (δ 190.6), C-3' (δ 160.9), C-4' (δ 179.8) and C-10' (δ 112.0); from H-6' (δ 7.67) to C-4', C-5' (δ 129.8), C-7' (δ 147.2), C-8' (δ 130.6), C-10' and C-12' (δ 20.5); from OH-9' (δ 12.5) to C-8', C-9' (δ 159.7) and C-10'; from H₃-11' (δ 3.92) to C-3'; and from H₃-12' (δ 2.21) to C-6' (δ 121.3), C-7' and C-8' indicated that xanthoradone A has another naphthoquinone skeleton. This was supported by the chemical shifts of two carbonyl carbons (C-1' (δ 190.6) and C-4' (δ 179.8)) derived from a quinone skeleton and the UV absorption (263 nm). Finally, the correlations observed between H₃-15 and OH-9', and between OH-10 and H₃-12' in

Table 2 ¹H- and ¹³C NMR chemical shifts of xanthoradones A and B

Position	Xanthoradone A		Xanthoradone B	
	δ _C	δ _H	δ _C	δ _H
1	171.5	—	171.5	—
2	76.8	4.79m	76.6	4.75m
3	34.7	ax: 3.00dd (<i>J</i> =17.0, 10.0 Hz) eq: 3.06dd (<i>J</i> =17.0, 4.0 Hz)	34.7	ax: 2.98dd (<i>J</i> =17.0, 10.0 Hz) eq: 3.05dd (<i>J</i> =17.0, 4.0 Hz)
4	133.5	—	133.3	—
5	116.1	6.99s	116.1	6.94s
6	140.0	—	140.3	—
7	98.1	6.71s	98.1	6.67s
8	160.3	—	160.8	—
9	109.0	—	106.4	—
10	154.7	—	155.2	—
10-OH	—	9.73s	—	9.69s
11	108.4	—	108.4	—
12	162.7	—	162.8	—
12-OH	—	13.9s	—	13.8s
13	99.7	—	99.5	—
14	20.7	1.57d (<i>J</i> =7.0 Hz)	20.7	1.53d (<i>J</i> =7.0 Hz)
15	56.0	3.84s	56	3.82s
1'	190.6	—	189.0	—
2'	109.6	6.08s	135.8	6.73q (<i>J</i> =2.0 Hz)
3'	160.9	—	148.3	—
4'	179.8	—	185.0	—
5'	129.8	—	133.0	—
6'	121.3	7.67s	163.2	7.33s
7'	147.2	—	163.6	—
8'	130.6	—	110.3	—
9'	159.7	—	161.1	—
9'-OH	—	12.5s	—	12.3s
10'	112.0	—	116.1	—
11'	56.6	3.92s	16.4	2.16d (<i>J</i> =2.0 Hz)
12'	20.5	2.21s	56.5	3.88s

Abbreviations: ax, axial; eq, equatorial.

Figure 2 Partial structure (a) and ¹H-¹H COSY and ¹³C-¹H HMBC experiments (b) of xanthoradone A.

Figure 3 ROESY experiments of xanthoradone A.

rotating frame nuclear overhauser effect spectroscopy (ROESY) experiments (Figure 3), and the long-range coupling of 4J observed from H-7 to C-8' in ^{13}C - ^1H HMBC experiments (Figure 2b) indicated that the two substructures of dihydronaphthopyranone and naphthoquinone are connected at C-9-C-8'. Taken together, the structure of xanthoradone A was elucidated as shown in Figure 1. The structure satisfied the degree of unsaturation and the molecular formula.

Structure elucidation of xanthoradone B

The molecular formula $\text{C}_{27}\text{H}_{22}\text{O}_9$ of xanthoradone B was the same as that of xanthoradone A. The ^1H NMR spectrum of xanthoradone B was almost identical to that of xanthoradone A except for a weak coupling constant ($J=2.0\text{ Hz}$) between H-2' and H-11' in xanthoradone B, which was not observed in xanthoradone A. In fact, cross peaks observed from H-2' (δ 6.73) to C-4' (δ 185.0), C-10' (δ 116.1) and C-11' (δ 16.4); from H-6' (δ 7.33) to C-4', C-5' (δ 133.0), C-7' (δ 163.6), C-8' (δ 110.3) and C-10'; from OH-9' (δ 12.3) to C-8', C-9' (δ 161.1) and C-10'; from H₃-11' (δ 2.16) to C-2' (δ 135.8), C-3' (δ 148.3) and C-4'; and from H₃-12' (δ 3.88) to C-7' in the ^{13}C - ^1H HMBC experiments indicated the presence of a naphthoquinone skeleton (Figure 4). This was also supported by the chemical shifts of two carbonyl carbons (C-1' (δ 189.0) and C-4' (δ 185.0)) derived from a quinone skeleton and the UV absorption (267 nm). Taken together, the structure of xanthoradone B was elucidated as shown in Figure 1; the oxygenated methyl group at R1 and the methyl group at R2 in xanthoradone A are reversed in xanthoradone B (Figure 1). The structure satisfied the degree of unsaturation and the molecular formula.

DISCUSSION

In this study, xanthoradones A and B were isolated from *Penicillium radicum* strain FKI-3765-2, and their planar structures were elucidated by NMR studies (Figure 1). They were found to have a common asymmetric biaryl skeleton containing a dihydronaphthopyranone and a naphthoquinone. Regarding the stereochemistry of xanthoradones, both xanthoradone A and B have one chiral carbon at C-2 in the structure. The orientation of the methyl group at C-2 of the δ -lactone in xanthoradone A was investigated by ^1H - ^1H spin decoupling experiments. On irradiation at H₃-14 (δ 1.57), a complex multiplet at H-2 (δ 4.79) became a simple doublet of doublets with $J_{2,3}=10.0$ and 4.0 Hz. The large coupling constant ($J_{2,3}=10.0\text{ Hz}$) indicated that H-2 and H_{ax}-3 (δ 3.00) are oriented in an axial-axial direction. This was supported by the coupling constants of H_{ax}-3 ($J=17.0, 10.0\text{ Hz}$). Thus, the methyl group at C-2 of the δ -lactone in xanthoradone A was determined to be equatorial in orientation as shown in Figure 1. Similarly, the orientation of the methyl group at C-2 of the δ -lactone in xanthoradone B was also deduced, as shown in Figure 1.

Figure 4 ^{13}C - ^1H HMBC experiments of xanthoradone B.

Some structurally related natural products, such as xanthoviridicatin D produced by *Penicillium viridicatum*³ and xanthoviridicatin E and F, as HIV-1 integrase inhibitors, produced by *Penicillium chrysogenum*,² have been reported previously (Figure 1). However, the binding pattern of the two substructures differs between xanthoradones and xanthoviridicatin; C-9 of a dihydronaphthopyranone moiety connects with C-8' of the aromatic site of the naphthoquinone moiety in xanthoradones, whereas the former connects with C-2' of the quinone site of the naphthoquinone moiety in known xanthoviridicatin.

By comparison with fungal biaryl compounds containing a dihydronaphthopyranone moiety, such as vioxanthin,⁴ rubrosulphin,⁵ viomellein⁵ and luteosporin,⁶ the absolute configuration of xanthoradones at C-2 can be deduced to be in 2R configuration. Furthermore, xanthoradones A and B have a chiral axis in the structures. The configuration of the axis might be *M* by comparing the CD spectra of (*P*) and (*M*)-vioxanthins;⁴ xanthoradones show a negative cotton effect at 281–289 nm and a positive cotton effect at 266–269 nm similar to (*M*)-vioxanthin.

METHODS

General experimental procedures

The UV spectra were recorded on a spectrophotometer (8453 UV-Visible spectrophotometer; Agilent Technologies, Santa Clara, CA, USA). IR spectra were recorded on a Fourier transform infrared spectrometer (FT-710; Horiba, Kyoto, Japan). Optical rotations were measured using a digital polarimeter (DIP-1000; JASCO, Tokyo, Japan). ESI-TOF-MS and HRESI-TOF-MS spectra were recorded on a mass spectrometer (JMS-T100LP; JEOL, Tokyo, Japan). Various NMR spectra were measured using a spectrometer (XL-400; Varian, Palo Alto, CA, USA).

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