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NOTE

Xanthoradone C, a new potentiator of imipenem activity against methicillin-resistant *Staphylococcus aureus*, produced by *Penicillium radicum* FKI-3765-2

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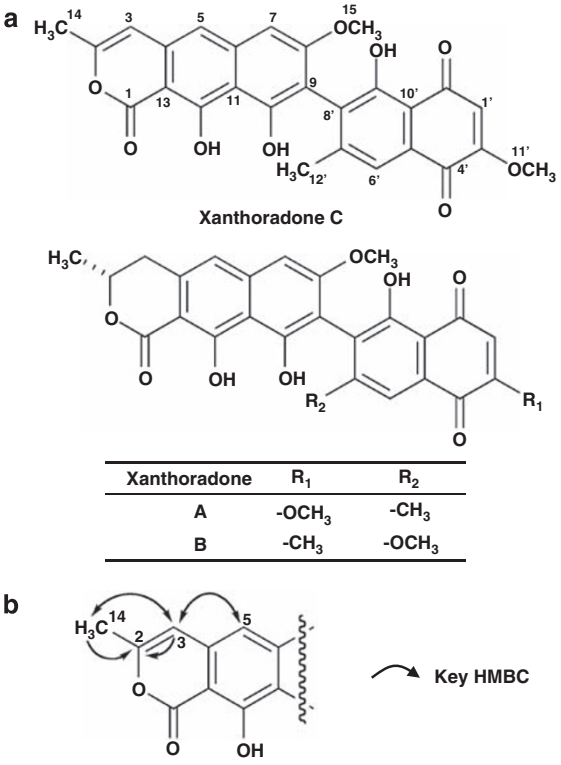
Xanthoradones A and B (Figure 1a) were isolated from the whole culture of *Penicillium radicum* FKI-3765-2 as potentiators of imipenem activity against methicillin-resistant *Staphylococcus aureus* (MRSA).[1,2] Further precise analysis of metabolites in the whole culture led to the discovery of a new congener named xanthoradone C (Figure 1a). In this study, the isolation, structure elucidation and biological properties of xanthoradone C are described.

P. radicum FKI-3765-2 was fermented as reported previously.[1,2] The 13-day-old whole culture (1500 g) was extracted with 3.0 l of acetone. After the acetone extracts were filtered and concentrated to remove acetone, we extracted the aqueous solution with ethyl acetate. The extracts were dried over Na_2SO_4 and concentrated *in vacuo* to dryness to yield a red brown material (2.5 g). The material was dissolved in 30% CH_3CN, applied to ODS column chromatography (150 g, SSC-ODS-7515-12; Senshu Scientific, Tokyo, Japan), and eluted stepwise with 30, 50, 70 and 100% CH_3CN containing 0.050% TFA (400 ml×2 tubes for each solvent). The second tube of 70% CH_3CN containing xanthoradones A to C was concentrated *in vacuo* to dryness to give a red brown material. The material (224 mg) was finally purified by preparative HPLC (column, Pegasil ODS, 20×250 mm; Senshu Scientific; solvent, 65% CH_3CN containing 0.050% TFA; detection, UV at 210 nm; flow rate, 8.0 ml min^{-1}). Under these conditions, xanthoradones A to C were eluted as peaks with retention times of 29.2, 31.6 and 39.6 min, respectively. The fractions were concentrated *in vacuo* to dryness to give pure xanthoradones A (13.7 mg), B (6.95 mg) and C (1.68 mg) as orange crystals.

Physicochemical properties of xanthoradone C are summarized in Table 1A. Xanthoradone C showed UV absorption maxima at 220, 282 and 360 nm, dissimilar to xanthoradones A and B (218 and 263–267 nm).[2] IR absorption at 3424 and 1629–1679 cm^{-1} suggested the presence of hydroxy and carbonyl groups in the structure.



Xanthoradone	R_1	R_2
A	$-OCH_3$	$-CH_3$
B	$-CH_3$	$-OCH_3$

Figure 1 (**a**) Structures of xanthoradones A to C. (**b**) ^{13}C-^{1}H HMBC experiments of xanthoradone C.

The molecular formula was determined to be $C_{27}H_{20}O_9$ on the basis of HRESI-TOF-MS measurement, indicating that xanthoradone C was smaller by two hydrogen atoms than xanthoradone A.[2] The ^{13}C NMR

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Table 1 (A) Physicochemical properties and (B) ^{1}H and ^{13}C NMR chemical shifts of xanthoradone C

	Xanthoradone C
(A)	
Appearance	Orange crystal
Molecular weight	488
Molecular formula	$C_{27}H_{20}O_9$
HRESI-TOF-MS (m/z)	
Calcd.:	487.1029 $(M-H)^-$
Found:	487.1040 $(M-H)^-$
UV (MeOH) λ_{max} nm (ε)	220 (10 800), 282 (22 500), 360 (3500)
$[\alpha]_D^{26}$	+264.4° (*c*=0.1, $CHCl_3$)
IR (KBr) ν_{max} (cm^{-1})	3424, 1679, 1629, 1540, 1506

	Xanthoradone C	
Position	δ_c	δ_H
(B)		
1	177.8	—
2	152.9	—
3	104.6	6.28s
4	132.4	—
5	112.4	7.07s
6	141.3	—
7	97.9	6.76s
8	160.6	—
9	109.0	—
10	154.5	—
10-OH		9.68s
11	108.4	
12	162.2	—
12-OH		13.6s
13	98.1	—
14	19.4	2.29s
15	56.0	3.86s
1′	190.6	—
2′	109.5	6.09s
3′	160.7	—
4′	179.8	—
5′	129.9	—
6′	121.3	7.67s
7′	147.3	—
8′	132.1	—
9′	159.7	—
9′-OH		12.5s
10′	112.0	—
11′	56.6	3.92s
12′	20.6	2.22s

spectrum (in $CDCl_3$) showed 27 resolved signals, which were classified into two methyl carbons, five sp^2 methine carbons, two oxygenated methyl carbons, nine sp^2 quaternary carbons, six oxygenated sp^2 quaternary carbons and three carbonyl carbons by analysis of the DEPT and HSQC spectra. The ^{1}H NMR spectrum (in $CDCl_3$) showed 20 proton signals, nine of which were suggested to be three hydroxy protons (δ 9.68, 12.5 and 13.6) and two oxygenated methyl protons (δ 3.86 and 3.92). These results supported the molecular formula. The connectivity of proton and carbon atoms was established by the ^{13}C-^{1}H HSQC spectrum (Table 1B). Comparison of the ^{1}H NMR spectra between xanthoradones A and C indicated that they share the same skeleton; however, the sp^3 oxygenated methine proton (H-2, δ 4.79) and the methylene protons (H_2-3, δ 3.00, 3.06) in xanthoradone A disappeared in xanthoradone C, and the sp^2 methine proton (δ 6.28) appeared in xanthoradone C. In addition, the methyl proton signal (H_3-15) changed to singlet from doublet and its chemical shift shifted to a lower field from δ 1.57 to δ 2.29.[2] Accordingly, it was concluded that xanthoradone C is 2,3-didehydro-xanthoradone A (Figure 1a). In fact, cross peaks were observed from H-3 (δ 6.28) to C-2 (δ 152.8), C-5 (δ 112.4) and C-14 (δ 19.4); from H-5 (δ 7.07) to C-3 (δ 104.6) and from H_3-14 (δ 2.29) to C-2 and C-3 in the ^{13}C-^{1}H HMBC experiments (Figure 1b). The structure satisfied the degree of unsaturation, UV and the molecular formula.

Furthermore, xanthoradone C has a chiral axis in the structure. The configuration of the axis might be *M* by comparing the CD spectra of (*P*)- and (*M*)-vioxanthins;[3] xanthoradone C show a negative cotton effect at 297 nm and a positive cotton effect at 278 nm as well as (*M*)-vioxanthin.[3]

Anti-MRSA activity of xanthoradone C was tested by the liquid microdilution method.[4] Xanthoradone C showed weak anti-MRSA activity with an MIC value of 8.0 μg ml^{-1}. Next, the potentiating effect of xanthoradone C on the activity of imipenem against MRSA was investigated according to the liquid microdilution method.[1,4] The concentration of xanthoradone C was set at 2.0 μg ml^{-1} for this experiment, which showed no effect on the growth of the MRSA K-24 strain.[1] Xanthoradone C showed moderate potentiating activity, reducing the MIC value of imipenem from 16 to 0.50 μg ml^{-1} (32-folds), whereas xanthoradones A and B were reported to enhance imipenem activity 266- to 533-fold.[1] Therefore, introduction of the double bond between C-2 and C-3 is unfavorable for the potentiation of imipenem activity against MRSA (Figure 1a).

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