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A Xanthanolide with Potent Antibacterial Activity against Methicillin-resistant *Staphylococcus aureus*

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Abstract

This study was conducted to find constituents of an annual herb, *Xanthium sibiricum* Patr er Widd, with effective antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA).

By monitoring antibacterial activity against MRSA strains, it was shown that a sesquiterpene lactone, identified as [3aR-(3aα,7β,8aβ)]-3,3a,4,7,8,8a-hexahydro-7-methyl-3-methylene-6-(3-oxo-1-butenyl)-2*H*-cyclohepta[b]furan-2-one, or xanthatin, isolated from leaves of the herb, had outstandingly potent activity against *S. aureus* species, including MRSA; its activity against MRSA and MSSA strains was similar. Other bacteria, e.g. *Staphylococcus epidermidis, Klebsiella pneumoniae, Bacillus cereus, Pseudomonas aeruginosa* and *Salmonella typhi*, were also susceptible at the concentrations tested but the compound had no inhibitory effect on some other bacteria, including *Escherichia coli*.

The results show that xanthatin has outstandingly potent activity against strains of *S. aureus* but that the activity of the compound is highly species-specific.

Methicillin-resistant strains of *Staphylococcus aureus* (MRSA) are resistant to essentially all β-lactam antibiotics. Many are also resistant to other antibacterial agents, including aminoglycosides (Brumfitt & Hamilton-Miller 1989; Cohen 1992; Neu 1992). Because of the relatively high frequencies of resistance of staphylococci to third-generation cephems (Ohno & Yamaguchi 1991; Matsumoto et al 1992), in hospitals where these drugs are used extensively the MRSA strains have a selective advantage in colonizing patients and spreading throughout the hospital (Brumfitt & Hamilton-Miller 1989; Cohen 1992). Hence the emergence of the MRSA strain poses a substantial threat to public health.

This study was performed to find novel antibacterial agents in natural products which are effective against MRSA. During the course of a screening test with various crude drugs, we found that a 50% ethanolic extract of the dry leaves of *Xanthium sibiricum* Patr er Widd showed strong antibacterial activity against MRSA. In this paper, we describe the antibacterial activity of an effective constituent of the herb.

Materials and Methods

Extraction and isolation

Extraction of target substances from the leaves of *X. sibiricum* was monitored by thin-layer chromatography (TLC) on precoated silica gel 60 F_{254} plates (Merck, Darmstadt, Germany) with chloroform–methanol, 9 : 1 (v/v) as mobile phase. Antibacterial activity against MRSA strains was examined by a disc-diffusion method.

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Pulverized leaves of the plant (3·6 kg), obtained from an herb garden of Alps Pharmaceutical Industries (Mukaimachi, Furukawa, Gifu, Japan), were extracted three times with 50% ethanol (36 L) under reflux for 2 h. The combined extracts were evaporated and partitioned with diethyl ether and butanol saturated with water. The fraction extracted in diethyl ether showed remarkable anti-MRSA activity. This fraction (24·09 g) was chromatographed on a 7 × 20 cm Wako gel C-300 column (Wako, Osaka, Japan) eluted stepwise with *n*-hexane, *n*-hexane-ethyl acetate, 9 : 1, and finally with ethyl acetate. Each fraction (500 mL) was collected and the active fractions (31–36; 15·74 g) were further separated on a 2·5 × 30 cm silica gel 60 column (Merck) with *n*-hexane-ethyl acetate, 1 : 1, as mobile phase. Each fraction (10 mL) was collected and fractions 11–60 were found to have potent anti-MRSA activity. These fractions were combined and further separated on a 4 × 7·5 cm ODS-Q3 (Wako) column eluted with methanol (50%, 80% and 100%). The anti-MRSA activity was detected in the 50%-methanol eluate. Final purification by HPLC on LiChrosorb Si 60 (2·5 × 25 cm; Merck) with *n*-hexane-ethyl acetate, 1 : 1, as mobile phase, afforded XS-1 (196 mg). Purity was monitored by TLC and by HPLC on LiChrosorb Si 60 (4·0 mm × 25 cm; Merck) with *n*-hexane-ethyl acetate, 1 : 1, as mobile phase and detection at 254 nm.

Preparation of bacteria

The MRSA and methicillin-sensitive *Staphylococcus aureus* (MSSA) strains listed in Table 1 and other bacteria listed in Table 2 were from laboratory stock cultures. The MRSA strains were defined on the basis of their resistance to methicillin and oxacillin, according to the guidelines of the National Committee for Clinical Laboratory Standards (1990). After culturing all strains on Mueller-Hinton agar (Difco, Detroit, MI), the cells were resuspended in Mueller-Hinton broth

Table 1. Antibacterial activity of methicillin and XS-1 against *Staphylococcus aureus*.

Bacteria	Minimum inhibitory concentration ($\mu g\,mL^{-1}$) Methicillin	XS-1
Methicillin-resistant *Staphylococcus aureus*		
1	250	7·8
2	> 250	7·8
3	250	15·6
4	> 250	15·6
5	> 250	7·8
6	250	7·8
7	> 250	7·8
8	> 250	15·6
9	> 250	15·6
10	31·3	7·8
11	> 250	7·8
12	> 250	7·8
13	> 250	7·8
14	> 250	15·6
15	> 250	15·6
16	> 250	7·8
17	15·6	7·8
18	31·3	15·6
19	31·3	7·8
20	3·9	7·8
Methicillin-sensitive *Staphylococcus aureus*		
21	2·0	7·8
22	2·0	7·8
23	2·0	15·6
24	2·0	15·6
25	2·0	7·8
26	2·0	7·8
27	2·0	7·8

(Difco) to give 10^8 colony-forming units mL^{-1}, and then incubated.

Determination of antibacterial activity

During the extraction and purification procedure, disc-diffusion tests were performed with Whatman AA discs (13·0 mm) containing various concentrations of extracts. The discs were placed on Mueller-Hinton agar, without NaCl supplementation, inoculated with 10^5 colony-forming units mL^{-1} of MRSA. The zone of inhibition was determined after incubation for 20 h at 37°C. To estimate the antibacterial activity of the purified isolate, the minimum inhibitory concentration (MIC) was determined according to the method of the Japanese Society for Antimicrobial Chemotherapy (1981) using Mueller-Hinton agar without added NaCl. The inoculum size of bacteria examined was adjusted to 10^6 colony-forming units mL^{-1}. Plates were read after 20 h incubation at 37°C.

Table 2. Antibacterial activity of XS-1 against Gram-positive and Gram-negative bacteria.

Bacterium	MIC ($\mu g\,mL^{-1}$)
Staphylococcus epidermidis IFO 3762	31·3
Bacillus cereus IFO 3514	62·5
Enterococcus faecalis ATCC 21212	> 250
Acinetobacter calcoaceticus ATCC 19606	> 250
Citrobacter freundii ATCC 8090	> 250
Enterobacter cloacae IFO 13535	> 250
Escherichia coli NIHJ JC-2	> 250
Klebsiella pneumoniae ATCC 10031	31·3
Pseudomonas aeruginosa NCTC 10490	125
Proteus mirabilis IFO 3849	> 250
Proteus vulgaris IID OX-19	> 250
Serratia marcescens IAM 1184	> 250
Salmonella typhi WO 961	125
Salmonella typhimurium IFO 13245	> 250

Results and Discussion

The 50% ethanolic extract of the leaves of *X. sibiricum* was partitioned between diethyl ether, butanol and water. Of these extracts only that in diethyl ether showed notable antibacterial activity against the MRSA strains. A combination of silica gel (Wako gel C-300, silica gel 60 and LiChrosorb Si 60) and reversed-phase (ODS-Q3) column chromatography of the diethyl ether extract afforded an anti-MRSA-active compound which was named XS-1 (yield from the dry leaves, 0·0054%).

The antibacterial activity of this compound and of the antibiotic methicillin as control is summarized in Tables 1 and 2. All the strains of MSSA examined were sensitive to methicillin (MIC 2·0 $\mu g\,mL^{-1}$), whereas MRSA strains were resistant to the antibiotic (MIC 3·9 to > 250 $\mu g\,mL^{-1}$). In contrast, XS-1 showed significantly potent activity against MRSA strains and against MSSA strains; no differences in susceptibility to the compound were detected between MSSA and MRSA strains (MIC 7·8–15·6 $\mu g\,mL^{-1}$). As shown in Table 2, whereas many of the strains examined were not inhibited by this compound, *Staphylococcus epidermidis* (MIC 31·3 $\mu g\,mL^{-1}$), *Klebsiella pneumoniae* (31·3 $\mu g\,mL^{-1}$), *Bacillus cereus* (62·5 $\mu g\,mL^{-1}$), *Pseudomonas aeruginosa* (125 $\mu g\,mL^{-1}$) and *Salmonella typhi* (125 $\mu g\,mL^{-1}$) were susceptible at the concentrations tested.

The genus Xanthium was chemically characterized by the occurrence of sesquiterpene lactones (McMillan et al 1975; Bohlmann et al 1981). The compound was identified by comparison with literature data for xanthanolides (Deuel & Geissman 1957; McMillan et al 1975; Kawazu et al 1979; Bohlmann et al 1981; Omar et al 1984).

XS-1 was obtained as colourless crystals, m.p. 103–105°C. The high-resolution mass spectrum showed a molecular ion peak at m/z 246 which corresponded to $C_{15}H_{18}O_3$. From spectral data, it was identified as [3aR-(3aα,7β,8aβ)]-3,3a,4,7,8,8a-hexahydro-7-methyl-3-methylene-6-(3-oxo-1-butenyl)-2*H*-cyclohepta[b]furan-2-one, xanthatin (Fig. 1; Deuel & Geissman 1957; McMillan et al 1975).

In the current study we have clearly demonstrated that the antibacterial activity of xanthatin was outstandingly potent against strains of *S. aureus*. In contrast, the compound had no inhibitory effect on the growth of several species of bacteria including *Escherichia coli*. Our data for *S. aureus* and *E. coli* are consistent with those reported by Tsankova et al (1994) and suggest that the activity of the compound is highly species-specific, i.e. specific to *S. aureus*.

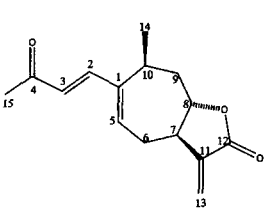

FIG. 1. The structure of xanthatin.

References

Bohlmann, F., Jakupovic, J., Schuster, A. (1981) Further eudesmanolides and xanthanolides from *Telekia speciosa*. Phytochemistry 20: 1891–1893

Brumfitt, W., Hamilton-Miller, J. (1989) Methicillin-resistant *Staphylococcus aureus*. N. Engl. J. Med. 320: 1188–1196

Cohen, M. L. (1992) Epidemiology of drug resistance: implications for a post-antimicrobial era. Science 257: 1050–1055

Deuel, P. G., Geissman, T. A. (1957) Xanthinin. II. The structure of xanthinin and xanthatin. J. Am. Chem. Soc. 79, 3778–3783

Japanese Society for Antimicrobial Chemotherapy (1981) Revised method for measuring minimum inhibitory concentration. Chemotherapy (Tokyo) 29: 76–79

Kawazu, K., Nakajima, S., Ariwa, M. (1979) Xanthumin and 8-epixanthatin as insect development inhibitors from *Xanthium canadense* Mill. Experientia 35: 1294–1295

Matsumoto, K., Tao, M., Iwagaki, A., Watanabe, K., Sakamoto, T. (1992) Methicillin-resistant *Staphylococcus aureus* (MRSA) infections: prevention and chemotherapy. Saishin-Igaku 47: 237–244

McMillan, C., Chavez, P. I., Plettman, S. G., Mabry, T. J. (1975) Systematic implications of the sesquiterpene lactones in the 'Strumarium' morphological complex (*Xanthium strumarium*, Asteraceae) of Europe, Asia and Africa. Biochem. Syst. Ecol. 2: 181–184

National Committee for Clinical Laboratory Standards (1990) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 2nd edn, Approved standard, NCCLS document M7-A2. National Committee for Clinical Laboratory Standard, Villanove, PA

Neu, H. C. (1992) The crisis in antibiotic resistance. Science 257: 1064–1073

Ohno, A., Yamaguchi, K. (1991) Infectious diseases caused by MRSA. I. From bacteriological viewpoint. Nippon Yakuzaishikai Zasshi 43: 1181–1188

Omar, A. A., Elrashidy, E. M., Ghazy, N. A., Metwally, A. M., Ziesche, J., Bohlmann, F. (1984) Xanthanolides from *Xanthium spinosum*. Phytochemistry 23: 915–916

Tsankova, E., Trendafilova, A. B., Kujumgiev, A. I., Galabov, A. S. (1994) Xanthanolides of *Xanthium italicum* Moretti and their biological activity. Z. Naturforsch. 49C: 154–155