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**Note**

# Isolation of an insecticidal compound oxalicine B from *Penicillium* sp. TAMA 71 and confirmation of its chemical structure by X-ray crystallographic analysis

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An insecticidal compound was isolated from *Penicillium* sp. TAMA 71 and showed 82% mortality against green peach aphid (*Myzus persicae*) at 100 ppm. The chemical structure of the compound was determined by X-ray crystallographic analysis to be identified as oxalicine B, and its NMR data were compatible with the chemical structure. A part of the known $^1$H NMR assignment was incorrect and was corrected. Insecticidal, miticidal and fungicidal tests were conducted at 500 ppm using three different insects, a mite and two plant pathogenic fungi besides the aphid to show 32% mortality and weak antifeeding activity against only western flower thrips (*Frankliniella occidentalis*) larvae. © Pesticide Science Society of Japan

*Keywords*: *Penicillium* sp., oxalicine, insecticidal activity, green peach aphid, western flower thrips.

## Introduction

In a series of our studies to search for pesticidal compounds from fungal sources,[1)] we isolated an insecticidal compound from solid state fermentation of *Penicillium* sp. TAMA 71 by bioassay-guided fractionation against green peach aphid (*Myzus persicae*). The chemical structure of the isolated compound was elucidated by X-ray analysis, and the compound was identified as oxalicine B (**1**) shown in Fig. 1. Oxalicine B (**1**) has already been isolated from *Penicillium oxalicum* and reported by Ubillas.[2)] The compound was reported again as a metabolite from *Penicillium thiersii* by Li *et al.*,[3)] and they determined its chemical structure and relative stereochemistry referring to the existing $^1$H and $^{13}$C NMR data of oxalicine A (**2**) and 15-deoxyoxalicine B (**4**) shown in Fig. 1; however, as it was found that part of the $^1$H NMR assignment reported was incorrect, we corrected it in this report.

Oxalicines[2–5)] including **1** and its related compounds, decaturins,[3,4)] is known to show antiinsectan activity in dietary assays against fall armyworm (*Spodoptera frugiperda*) and to cause a reduction in the growth rate. As we were also interested in the biological activity of **1**, insecticidal, miticidal and fungicidal tests against three different insects, a mite and two plant pathogenic fungi besides the aphid, were conducted at 500 ppm.

## Materials and Methods

The fungus *Penicillium* sp. TAMA 71 was isolated from a soil sample collected in Kamakura-yama, Kamakura, Kanagawa Prefecture, Japan, and assigned as *Penicillium* sp. of subgenus *Furcatum* in the genus *Penicillium* based on morphological characteristics such as good growth on Glycerol-25%-nitrate agar (G25N), biverticilate penicilli with divergent metulae, ampulliform phialides, and smooth-walled globose conidia in a long chain.[6)]

Isolation of **1** was conducted according to the following procedure. To a well-grown slant culture of *Penicillium* sp. TAMA 71

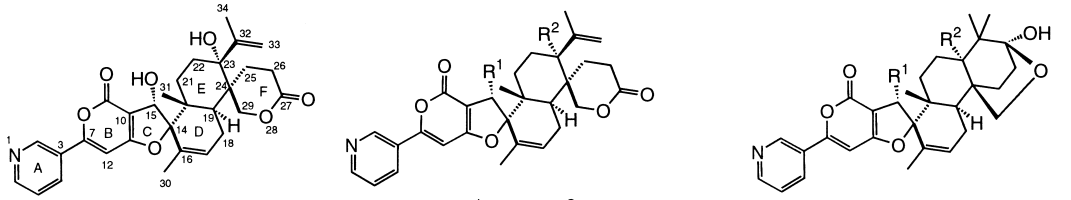


**Fig. 1.** Chemical structures of oxalicine B (**1**) and related compounds.

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was added 4 ml of sterile water to prepare a conidial suspension. One milliliter of the suspension thus prepared was inoculated into a production medium consisting of 10 g of rolled barley, 20 mg of yeast extract, 10 mg of sodium tartrate, 10 mg of $KH_2PO_4$ and 10 ml of $H_2O$ in 250 ml Erlenmeyer flasks (40 flasks in total). Fermentation was conducted for 12 days at 25°C under stationary conditions. The 40 flasks of solid cultures were extracted with 1000 ml of 1-butanol, and the resulting butanolic extract (400 ml) was filtered and concentrated *in vacuo*. The concentrated extract was 2244.7 mg in weight and showed 65% mortality against the aphid at 1000 ppm. The concentrated extract was partitioned with ethyl acetate (EtOAc) and water, and the active EtOAc-soluble part was fractionated repeatedly by silica gel column chromatography. The more active sample thus obtained was fractionated by liquid chromatography (LC) to give a fraction mainly containing compound **1**. The $t_R$ of the fraction was from 11.4 min to 14.1 min, and the conditions for the LC were as follows: column: Tosoh TSKgel ODS-80Ts 21.5 i.d.×300 mm; wave length: 254 nm; column temperature: room temperature; mobile phase: $CH_3CN$–$H_2O$ [48/52 (v/v)]; flow rate: 10 ml/min; sample solvent: $CH_3CN$; sample volume: 1.0 ml; sample concentration: 8640 ppm. After LC, the active fraction was chromatographed again in a silica gel column to give a solid, which was recrystallized three times successively from a mixture of EtOAc and Hex, EtOAc and a mixture of EtOAc and MeOH to finally obtain 9.6 mg of pure **1** as a white solid. The $R_f$ value of the pure **1** on TLC using EtOAc as a developing solvent was 0.4, and the spot was colored by molybdophosphoric acid. Pure **1** was obtained in 0.56% yield from the concentrate of the butanol extract. For X-ray crystallographic analysis, a colorless columnar crystal of **1** was prepared by recrystallizing from EtOH, and a Rigaku RAXIS imaging plate area detector with graphite monochromated Mo-K$\alpha$ radiation ($\lambda$=0.71069 Å) was used. Crystal data were collected at 23±1°C and are as follows: crystal color, habit: unknown, unknown; crystal dimensions: 0.10×0.10×0.10 mm; crystal system: orthorhombic; lattice parameters: a=6.4740 (1) Å, b=10.3950 (1) Å, c=38.2195 (4) Å, V=2572.07 (5) Å$^3$; space group: $P2_12_12_1$ (#19); Z value: 4; $D_{calc}$: 1.342 g/cm$^3$; $F_{000}$: 1104.00; m (MoK$\alpha$): 0.95 cm$^{-1}$. The conditions for intensity measurements are as follows: $2q_{max}$: 55.0°; No. of reflections measured: total 20,411, unique 3409 ($R_{int}$=0.031); corrections: Lorentz-polarization, absorption (trans. factors: 0.9780–1.0244). The structure solution and refinement are as follows: structure solution: direct methods (SIR 92); refinement: full-matrix least-squares on $F^2$; function minimized: Sw $(Fo^2–Fc^2)^2$; least squares weight: $1/[0.002Fo^2+1.000s^2 (Fo)]/(4Fo^2)$; No. observations [I>–10.00s (I)]: 3391; No. variables: 375; residuals R1, wR2: 0.040, 0.107; goodness of fit indicator: 0.90. The other physicochemical data of **1** are as follows: mp (EtOH): 268–271°C (dec.); $[\alpha]_D^{26}$ +145° (*c* 0.0544, $CHCl_3$); UV $\lambda_{max}$ ($CHCl_3$, 26°C) nm ($\varepsilon$): 245 (14200), 272 (8260), 331 (10800); IR $\nu_{max}$ (KBr) cm$^{-1}$: 3400 (br. m), 2960 (w), 1760 (m), 1750 (m), 1720 (s), 1640 (w), 1580 (w), 1480 (w), 1460 (w); ESIMS *m/z*: 520 $(M+H)^+$, 561 $(M+CH_3CN+H)^+$; HRESIMS *m/z* $(M+H)^+$: Calcd. for $C_{30}H_{34}O_7N$: 520.2335, Found: 520.2409; HRESIMS *m/z*

**Table 1.** $^1$H NMR (500 MHz) and $^{13}$C NMR (125 MHz) data for oxalicine B (**1**)[a]

| Position | $^{13}$C ($\delta$) | | $^1$H ($\delta$) | |
|---|---|---|---|---|
| 2 | 147.11 | CH | 9.02 | br. d, *J*=1.8 Hz |
| 3 | 127.30 | C | | |
| 4 | 133.35 | CH | 8.09 | ddd, *J*=8.1, 1.7, 1.7 Hz |
| 5 | 123.75 | CH | 7.42 | dd, *J*=8.1, 4.8 Hz |
| 6 | 151.95 | CH | 8.71 | br. d, *J*=3.8 Hz |
| 7 | 161.75 | C | | |
| 9 | 170.76 | C | | |
| 10 | 105.77 | C | | |
| 11 | 160.29 | C | | |
| 12 | 94.02 | CH | 6.69 | s |
| 14 | 101.10 | C | | |
| 15 | 74.24 | CH | 5.52 | br. d, *J*=4.4 Hz |
| 16 | 130.70 | C | | |
| 17 | 129.94 | CH | 5.81 | br. d, *J*=5.3 Hz |
| 18 | 24.54 | $CH_2$ | 2.25–2.40 | m |
| | | | 2.16 | br. ddd, *J*=18.1, 4.7, 4.7 Hz |
| 19 | 43.56 | CH | 2.66 | dd, *J*=12.7, 4.7 Hz |
| 20 | 41.04 | C | | |
| 21 | 24.49 | $CH_2$ | 2.42–2.48 | m |
| | | | 2.25–2.40 | m |
| 22 | 29.25 | $CH_2$ | 2.25–2.35 | m |
| | | | 1.45 | br. dd, *J*=14.0, 3.5 Hz |
| 23 | 76.17 | C | | |
| 24 | 44.56 | C | | |
| 25 | 25.98 | $CH_2$ | 2.53 | ddd, *J*=14.1, 14.1, 4.9 Hz |
| | | | 1.54–1.64 | m |
| 26 | 29.93 | $CH_2$ | 2.42–2.48 | m |
| | | | 2.30–2.40 | m |
| 27 | 173.48 | C | | |
| 29 | 67.79 | $CH_2$ | 4.52 | d, *J*=12.7 Hz |
| | | | 4.41 | d, *J*=12.7 Hz |
| 30 | 19.45 | $CH_3$ | 1.61 | s |
| 31 | 15.91 | $CH_3$ | 1.31 | s |
| 32 | 150.66 | C | | |
| 33 | 115.09 | $CH_2$ | 5.21 | s |
| | | | 5.08 | s |
| 34 | 21.67 | $CH_3$ | 1.91 | s |
| 15-O*H*[b] | | | 3.23 | br. d, *J*=4.4 Hz |
| 23-O*H*[b] | | | 1.36 | s |

[a] NMR spectra were measured using $CDCl_3$ containing 0.03% tetramethylsilane as a solvent. [b] The existence of hydroxyl groups was confirmed by $D_2O$ exchange experiment.

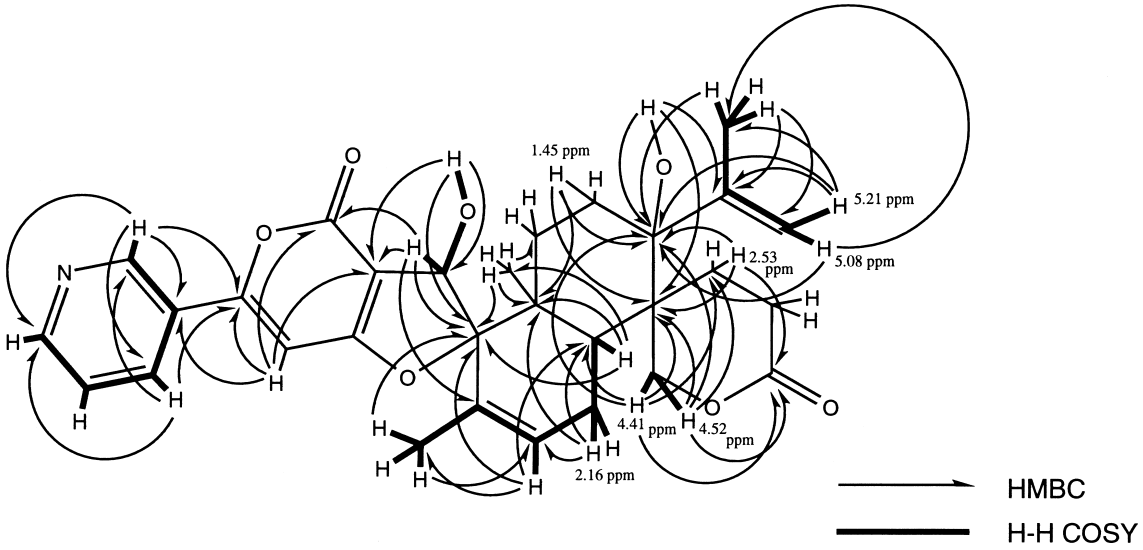


**Fig. 2.** C–H correlations from the HMBC spectrum and H–H correlations from the COSY spectrum of oxalicine B (**1**).

$(M+CH_3CN+H)^+$: Calcd. for $C_{32}H_{37}O_7N_2$: 561.2601, Found: 561.2599; $^1H$ NMR and $^{13}C$ NMR data are shown in Table 1.

The insecticidal activity of **1** against green peach aphid (*Myzus persicae*) was evaluated at two doses, 200 and 100 ppm. The insecticidal, miticidal and fungicidal activities of **1** against three different insects, diamondback moth (*Plutella xylostella*) larvae, western flower thrips (*Frankliniella occidentalis*) larvae and silverleaf whitefly (*Bemisia argentifolii*) larvae, a mite two-spotted spider mite (*Tetranychus urticae*) and two plant pathogenic fungi powdery mildew (*Sphaerotheca fuliginea*) and gray mold (*Botrytis cinerea*) were also evaluated at 500 ppm. The insecticidal and miticidal tests were conducted in the same manner as described in our previous reports,[1,7] and fungicidal tests were performed in the same manner as described in other unexamined patent publications.[8,9]

## Results and Discussion

We tried to elucidate the chemical structure of **1** on the basis of spectroscopic data. The HRESIMS analysis of **1** gave the molecular formula $C_{30}H_{33}O_7N$, and the formula was supported by $^1H$ NMR and $^{13}C$ NMR data. The signals of methyl, methylene, methine and quarternary carbons in the $^{13}C$ NMR spectrum were confirmed by DEPT spectra. The mode of binding between proton and carbon atoms, and the relationships among proton atoms were elucidated by HMQC and COSY spectra, respectively. The ring system consisting of D, E, and F rings was determined by HMBC spectrum, but the bicyclic moiety consisting of B and C rings was not (Fig. 2).

The chemical structure of **1** was not determined by NMR analysis, but by X-ray analysis. ORTEP drawing of the analyzed

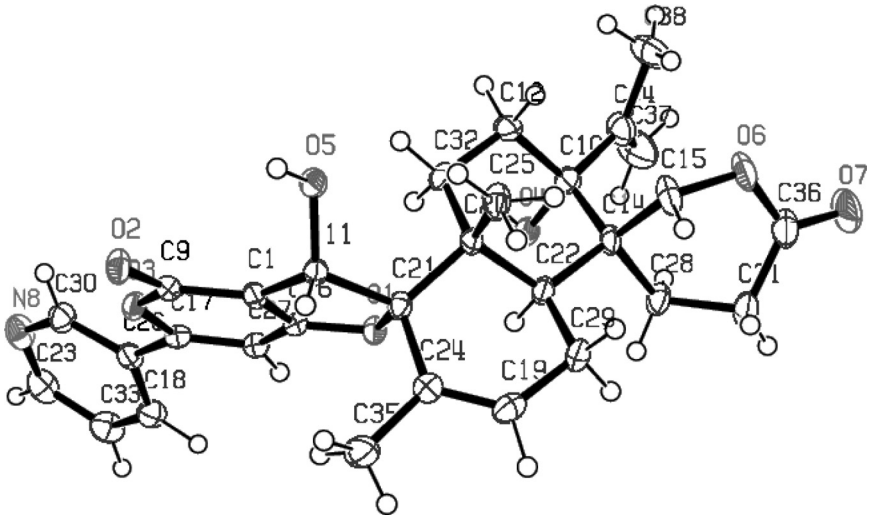


**Fig. 3.** ORTEP drawing of oxalicine B (**1**).

compound is shown in Fig. 3, and revealed the chemical structure and relative stereochemistry of **1**.

Our NMR data of **1** were correctly assigned to proton and carbon atoms of the structure determined by X-ray analysis. The assignments of $^{1}$H NMR and $^{13}$C NMR spectra of **1** are shown in Table 1. Then, the spectroscopic data and supporting information reported by Li *et al.*[3] were compared with ours. Their assignments of $^{13}$C NMR spectrum agreed with ours; however, as there were four incorrect assignments in their $^{1}$H NMR data, we corrected them as described below. A broad doublet at 1.22 ppm assigned to the proton atom at 22 position was not a signal of the proton atom of **1**, and the doublet was not observed in our spectrum. A double-double-doublet signal at 1.42 ppm assigned to the proton atom at 21 position should be reassigned to the proton atom at 22 position. A multiplet at 2.42 ppm assigned to the proton atom at 18 position should be reassigned to the proton atom at 21 position. An omitted signal was a multiplet at about 2.30 ppm assigned to the proton atom at 18 position in our data.

Compound **1** showed 87% and 82% mortality against the aphid at 200 ppm and 100 ppm, respectively. The compound also showed 32% mortality and weak antifeeding activity against thrips at 500 ppm, but did not show any activities against the others in the insecticidal, miticidal and fungicidal tests. It is interesting to clarify the structure–activity relationships of the compounds shown in Fig. 1 to obtain more active compounds.

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**References**

1) M. Abe, T. Imai, N. Ishii, M. Usui, T. Okuda and T. Oki: *Biosci. Biotechnol. Biochem.* **69**, 1202–1205 (2005).
2) R. P. Ubillas: "Chemical Studies on *Penicillium oxalicum*," Ph. D. thesis, University of Missouri-Columbia, pp. 61–74, 1990.
3) C. Li, J. B. Gloer, D. T. Wicklow and P. F. Dowd: *J. Nat. Prod.* **68**, 319–322 (2005).
4) Y. Zhang, C. Li, D. C. Swenson, J. B. Gloer, D. T. Wicklow and P. F. Dowd: *Org. Lett.* **5**, 773–776 (2003).
5) R. P. Ubillas, C. L. Barnes, H. Gracz, G. E. Rottinghaus and M. S. Tempesta: *J. Chem. Soc. Chem. Commun.* **1989**, 1618–1619.
6) J. I. Pitt: "A Laboratory Guide to Common *Penicillium* Species," Food Science Australia, North Ryde, Australia, 2000.
7) M. Abe, T. Imai, N. Ishii and M. Usui: *Biosci. Biotechnol. Biochem.* **70**, 303–306 (2006).
8) H. Miyake, M. Sasaki, T. Imai and N. Umetsu (Otsuka Chemical Co., Ltd.): *Jpn. Kokai Tokkyo Koho JP* 2004-269402 (2004) (in Japanese).
9) K. Manabe, T. Komura and K. Sagayama (Otsuka Chemical Co., Ltd.): *Jpn. Kokai Tokkyo Koho JP* 10-195049 (1998) (in Japanese).