Synthesis and Antifungal Activity of New 3,4,7-Trisubstituted Coumarins

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Fifty-three new 3-(2-diethylaminoethyl)-4-methyl-7-substituted coumarins were synthesized by four different routes utilizing commercially available 3-(2-diethylaminoethyl)-7-hydroxy-4-methylcoumarin hydrochloride. Their antifungal activities were measured against a phytopathogenic fungi, Botrytis cinerea. Some compounds with a substituted benzoyloxy, benzoxoxyethoxy, methylbenzoylaminooxy, or benzoanilinophenox group at the 7-position had an inhibitory effect (MIC: <50μm) on the germination of spores in an in vitro screening system. Among them, eight derivatives had interest activities. The highest level of activity (MIC: 7.8μm) was observed for the coumarin with a 7-[4-(2,4,6-trichlorobenzoyloxy) phenoxy] substitution. This derivative also inhibited the germination of four other plant pathogens, Collectotrichum orbiculare, Alternaria malu, Phytophthora capsici, and Pyricularia oryzae. © Pesticide Science Society of Japan

Keywords: coumarin, antifungal, fungicide, spore germination inhibitor.

INTRODUCTION

Many useful fungicidal compounds have a hetero ring with nitrogen atoms, such as an azole ring. However, the application of compounds having a hetero ring containing only oxygen atoms is still limited. Fenfuram, furcarbanil, and methfuram with a furan ring, furconazole with a hydrofuran ring, and azaconazole and propiconazole with a dioxolan ring are considered representative of synthetic fungicides. Among natural products, ambruticin, which has dihydro- and tetrahydro-pyranyl rings, showed excellent antifungal activity against Botrytis cinerea (BC) [minimum inhibitory concentration (MIC): 50ppb, Fujimaki and Kochi, unpublished] but has not been adopted for agriculture usage because of its instability in the field. Therefore, we screened various commercialized compounds and synthetic intermediates, bearing a pyran ring, in an attempt to develop a new class of fungicides, and found that 3-(2-diethylaminoethyl)-7-hydroxy-4-methylcoumarin (I) showed positive activity (MIC: 500ppm, Fujimaki and Kochi, unpublished). Although it has been reported that streptonivacin bearing a 3,4,7,8-tetrasubstituted coumarin moiety exhibited antibacterial activity, to the best of our knowledge no antifungal coumarins have been reported. Therefore, the structural modification of compound I was carried out to improve its activity. This paper deals with the synthesis and antifungal evaluation of new derivatives produced by esterification or ether formation at the 7-hydroxy group of I, taking into consideration the long molecular shape of ambruticin.

MATERIALS AND METHODS

1. Analytical Instruments

Melting points (Mp) were measured with a Büchi 535 melting point apparatus, and were uncorrected. 1H NMR spectra were recorded on a JEOL JNM-ER500 spectrometer at 500 MHz using tetramethylsilane (TMS) as an internal standard. Electrospray ionization-mass spectra (ESI-MS) were measured on a Finnigan LCQ/IT mass spectrometer.

2. Synthesis

2.1. Benzoyl esters of I (I, Route 1 in Fig. 1)

2,4,6-Trichlorobenzoyl chloride was added into a mixture of I (hydrochloride, Aldrich Co., Ltd.) and potassium carbonate dissolved in acetonitrile with stirring at room temperature. After warming for 4 hr at 50°C, benzoyl ester (2, I, X=2,4,6-Cl3) as a colorless crystal was obtained by filtration in 64% yield. Compound 2 [X=3-(2,4,6-Cl3,Ph−CONH)] and the other five esters (I, X=H, 3-Me, 3-(3-MePh−CONH), 3-(2,4,6-Cl3,Ph−CONH), (E)-4-(3-MePh−COCH=CH)] were synthesized using the same procedure.

2.2. 3-(2-Diethylaminoethyl)-4-methyl-7-(substituted benzoxoxyethoxy)coumarins (III, Route 2 in Fig. 1)

By dropwise addition of triethylamine (TEA) in an ice-cold bath, m-toloyl chloride was coupled with 2-iodoethanol in tetrahydrofuran (THF) to yield 1-(3-methylbenzoxoxy)-2-idoethane (II, X=3-Me). The iodo-compound was treated with a mixture of I and K2CO3 in acetonitrile under refluxing to make the ether 4 (III, X=3-Me), which was purified by silica gel column chromatography using EtOAc mixed with TEA (200:4). Using the same procedure, the other thirty-one 7-(substituted benzoxoxyethoxy)coumarins (III), including 5–7 (see structures in Table 1), were systematically prepared from the substituted benzoyl chlorides (including X=2-Me, 4-Me, 2-F, 3-F, 4-F, 2-Cl, 3-Cl, 4-Cl, 3,4-Cl2, 3,5-Cl2) with an overall yield of ca. 60%.
Fig. 1. Chemical structure of 1 (Cm-OH) and synthesis routes of 2–9, which were obtained by modifying the 7-hydroxy group of 1. Route 1: route for derivatives 1 (2 and 3 and four other compounds), Route 2: route for derivatives 4 (5–7 and twenty-seven other compounds), Route 3: route for derivatives 6 (8 and two other compounds), and Route 4: route for derivatives 9 (9 and twelve other compounds). a: TEA/THF, b: K₂CO₃/CH₃CN, c: NaH/DMF, d: H₂NNH₂/ETHOH, e: WSC/CH₂Cl₂, f: H₂/Pd-C/EthOH.

2.3. 3-(2-Diethylaminoethyl)-4-methyl-7-(substituted benzoylaminoethoxy)coumarin (vi, Route 3 in Fig. 1) Dimethylformamide was added drop-wise to a mixture of N-(2-bromoethyl)phthalimide, I, and NaH under argon gas. After stirring at 90°C, 7-(2-phthalimideethoxy)coumarin (iv) was extracted with EtOAc and purified by silica gel column chromatography using EtOAc mixed with TEA (200:4). The phthalic moiety of iv was removed by refluxing with anhydrous hydrazine in ethanol. Filtration of deposited solids yielded 3-(2-aminoethyl)coumarin (v), which was coupled with m-toluic acid using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSC) as a carboxy group activating reagent in CH₂Cl₂ to convert it into 8 (vi, X = 3-Me). After column chromatography using EtOAc mixed with TEA (200:4), purified 8 was obtained in a 1.7% overall yield from 1. Two other derivatives (vi) were prepared by the reaction of compound v with benzoic acids substituted differently (X = 2,4,6-Cl₃ and 2-EtOC₂H₅).

2.4. 3-(2-Diethylaminoethyl)-4-methyl-7-[4-(substituted benzoylamino-phenoxy)coumarin (ix, Route 4 in Fig. 1) A mixture of 4-fluorornitrobenzene, compound I, and K₂CO₃ in acetonitrile was stirred under refluxing to yield 7-(4-nitrophenoxy)coumarin (vii), which was hydrogenated to 7-(4-aminophenoxy)coumarin (viii) over palladium carbon in EThOH at room temperature. Acylation of the amino group produced 9 (ix, Y = 2,4,6-Cl₃) in the same manner as the esterification of 1 with 2,4,6-trichlorobenzoyl chloride in Route 1. Based on 1, the overall yield of 9 was ca. 80%. Using the same procedure, five other 4-(substituted benzoylamino)phenoxy derivatives (ix) were pre-
pared by the reaction of compound viii with benzoyl chlorides substituted differently (including Y=2-Me, 3-Me, 4-Me, 3-(3-MePhCONH), 3-(2,4,6-Cl,PhCONH)). Starting from 2-fluoronitrobenzene, two 2-(substituted benzoylamino)phenoxy derivatives were synthesized via 7-(2-aminophenoxy)coumarin.

3. Evaluation of Antifungal Activities

The antifungal activity of the newly synthesized coumarins was assayed in 96-well microplates (Kochi, unpublished) using a sterile 2-fold diluted potato dextrose broth (Difco Co.). Spores of BC, Colletotrichum orbiculare (CO), Alternaria mali (AM), Phy
tophthora capsici (PC), and Pyricularia oryzae (PO) were suspended in the broth (1×10^5 spores/ml) and added to 3,4,7-trisubstituted coumarin dissolved in dimethyl sulfoxide (DMSO) at various concentrations (2.0–500 ppm). A portion of 0.1 ml of each spore suspension was added to a well of the microplate and incubated at 25°C in darkness. The growth of the fungi was assessed visually four days later, and antifungal activity was expressed as the MIC (the minimum concentration showing inhibition). The tests were repeated twice.

### Table 1. Chemical properties of 3-(2-diethylaminoethyl)-4-methyl-7-substituted-coumarins (2–9, Cm–OR) synthesized by four different routes starting from 1 (Cm–OH).

<table>
<thead>
<tr>
<th>Comp. #</th>
<th>R</th>
<th>Mp (°C)</th>
<th>ESI-MS (m/z)</th>
<th>NMR of R (CDCl₃, TMS, δ ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2,4,6-Cl,PhCO</td>
<td>152.9–153.9</td>
<td>488 (7), 486 (31), 484 (87), 482 (100) [M+1]^+], 413 (11), 411 (36), 409 (28)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3-(2,4,6-Cl,PhCONH)–PhCO</td>
<td>163.2–166.1</td>
<td>438 (100) [M+1]^+], 365 (28)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>3-MePhCOOC(CH₃)–H</td>
<td>90.7–91.6</td>
<td>438 (100) [M+1]^+], 365 (28)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2,4,6-Cl,PhCOOC(CH₃)H₂</td>
<td>65.9–68.7</td>
<td>437 (100) [M+1]^+], 276 (19), 203 (50)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>(E)-4-(3-MePhCOCH=CH)–PhCOOC(CH₃)H₂</td>
<td>98.3–102.8</td>
<td>437 (100) [M+1]^+], 276 (19), 203 (50)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>3-(3-MePhCONH)Ph–COOC(CH₃)H₂</td>
<td>123.5–125.9</td>
<td>437 (100) [M+1]^+], 276 (19), 203 (50)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>3-MePhCONHC(CH₃)H₂</td>
<td>80.8–85.5</td>
<td>437 (100) [M+1]^+], 276 (19), 203 (50)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>4-(2,4,6-Cl,PhCONH)Ph–COOC(CH₃)H₂</td>
<td>260.0–265.3</td>
<td>579 (3), 577 (33), 575 (89), 573 (100) [M+1]^+], 504 (4), 502 (11), 500 (9)</td>
<td></td>
</tr>
</tbody>
</table>

Cm–O– of 4

\[
\begin{align*}
\text{at} & \text{J}=8.5 \text{Hz},
\end{align*}
\]
RESULTS AND DISCUSSION

Fifty-three new coumarin derivatives were synthesized by Routes 1–4 in Fig. 1 utilizing commercially available 3-(2-diethylaminomethyl)-7-hydroxy-4-methylcoumarin (1) as a starting material. The structure of each compound was confirmed from its NMR and ESI-MS data. Table 1 shows the melting points, NMR data, and ESI-MS data of four representatives (2, 4, 8, and 9) prepared via different routes. 1H and 13C signals of 4 were assigned based on two-dimensional experiments, and NMR data of all other derivatives, which were analyzed in comparison with the assignment for 4, confirmed their partially modified substituent at the 7-position of 1. ESI-MS measurements in the mode for positive ions showed [M+1]' as a base peak, based on which molecular formulae and numbers of chlorine atoms in the chlorinated derivatives were estimated. Additionally, all derivatives synthesized by Routes 1, 2, and 4 produced characteristic fragment ions at m/z M–73, probably losing NEt3+H in the substitution at the 3-position. Compounds synthesized by Route 3 exhibited other ions at m/z 276 after cleavage of the substituent at the 7-position and at m/z 203 (276–73) resulting from the loss of NEt3+H.

Every coumarin synthesized in this study inhibited the germination of BC spores more effectively than the parent 1, indicating that the modification of the 7-hydroxy group was effective in generating antifungal activity. As shown in Table 2, while the activity exhibited by the derivatives with a benzyl amide moiety, such as 8, which are synthesized by Route 3, showed small increases, a more effective derivatization was found in the compounds synthesized by other routes. Notably, the MIC values of 7 and 9, the strongest inhibitors of BC tested in this study, were ca. 60 times smaller than the MIC of 1. This result indicates that the effect of the substitution at the 7-position is not dependent on the type of linkage, such as an ester or ether bond. Generally, compounds with a 2,4,6-trichlorobenzyl moiety effectively inhibited the germination of BC, and 5, which has this moiety, was the strongest inhibitor among many benzoyloxytetrahydrocoumarins (iii) having only one benzene ring in the 7-substitution. The activity of the derivatives iii was also dependent on the length of the 7-substitution, and derivatives bearing two benzene rings, such as 6 and 7, were more active than those with one ring substituent, such as 4. Furthermore, the inhibitory activities of eight selected coumarins, 2–9, were measured against four phytopathologic fungi, CO, AM, PC, and PO. The results are also shown in Table 2. Compound 9 most effectively inhibited the germination of the former three fungi among the coumarins tested, but its effects on these fungi, particularly CO, were weaker than its effect on BC. In the case of the other compounds, similarly, a higher concentration is necessary to inhibit the germination of the three fungi than to inhibit BC, except in the case of the AM spores and 8. It has been reported that O-ethoxyethyl-N-[2-(4-trifluoromethoxyphenoxy)ethyl] salicylic amide expresses strong activity. But compounds 8 and 9 (X=2-BrOC2H5OPh), which have the same O-ethoxyethyl-N-[2-(4-trifluoromethoxyphenoxy)ethyl] salicylic amide were less active. A comparison of the activities of 4 and 8 indicated that the amide linkage connected to the coumarin part reduced the level of activity against all fungus tested. Although 6 was remarkably active against only BC, 7, its structurally related derivative, had a rather wide antifungal spectrum. In the case of PO, the germination was most strongly inhibited by 2. Interestingly, this activity is stronger than that against the BC spores.

In conclusion, we selected 9, which has the optimized 7-substitution pattern of 1, as a lead for new fungicides. In order to advance to the level of field applications, however, further modifications are necessary to increase the activity by more than one

Table 2. Antifungal activity of new synthetic 3-(2-diethylaminomethyl)-4-methyl-7-substituted-coumarins (2–9, Cm–OR, R=X–Ph–Z–) against five phytopathologic fungus (BC, CO, AM, PC, and PO)

<table>
<thead>
<tr>
<th>Comp. #</th>
<th>Synthetic route</th>
<th>Substitution at 7-position (R)</th>
<th>MIC values (ppm)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>X</td>
<td>Z</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>—</td>
<td>H</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>2,4,6-Cl3</td>
<td>CO–</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>3-(2,4,6-Cl2Ph–CONH)</td>
<td>CO–</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>3-Me</td>
<td>COOC2H5–</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>2,4,6-Cl3</td>
<td>COOC2H5–</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>(E)-4-(3-MePh–CH=CH)</td>
<td>COOC2H5–</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>4-(3-MePh–CONH)</td>
<td>COOC2H5–</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>3-Me</td>
<td>CONHC2H5–</td>
</tr>
<tr>
<td>9</td>
<td>4</td>
<td>4-(2,4,6-Cl2Ph–CONH)</td>
<td>—</td>
</tr>
</tbody>
</table>

*BC: Botrytis cinerea, CO: Colletotrichum orbiculare, AM: Alternaria mali, PC: Phytophthora capsici, PO: Puccinia oryzae.*
order of magnitude. The structural improvement of 9 and an understanding of its mode of action would be important future subjects.

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REFERENCES