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Synthesis and Biological Activities of Isocoumarins

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Four series of isocoumarin derivatives, 3–acetoxy–4–acetylisocoumarins, 3–methylisocoumarins, 4–ethoxycarbonyl–3–methylisocoumarins and 4–carboxyisocoumarins were synthesized from homophthalic acids and related compounds. The biological activities of these isocoumarins on radish, rice, barnyard grass and *Asp. niger* were examined. Three isocoumarins, 4–carboxy–6–chloro–, 4–carboxy–7–chloro– and 4–carbethoxy–6–chloro–3–methylisocoumarins showed phytotoxic effect on radish and rice at 100 ppm, but they stimulated the root elongation of radish and rice at lower concentrations. 4–Carbethoxy–6,7–dimethoxy–3–methylisocoumarins also indicated a similar effect on radish, but it scarcely affected the rice growth. Barnyard grass were less sensitive than rice and radish to such isocoumarins. 3,6–Dimethyl– and 6–methoxy–3–methylisocoumarins inhibited the germination of spores of *Asp. niger*.

INTRODUCTION

A lot of isocoumarins have been obtained from plants and fungi as secondary metabolites and some of them possess very interesting bioactivities. For example, 5–methylmellein obtained from *Fusicoccum amygdari* Del. inhibits the germination of spores of fungi, but it is not phytotoxic.[1] 6–Methoxymellein, which is produced by a carrot inoculated with fungi, is thought to be a phytoalexin.[2–6] 6–Methoxymellein was also isolated from a fungi *Sporormia bipartis* Cain[7] and *Sporomia affinis* Sacc.[8] Sclerin, sclerotinin A and B promote remarkably both germination and elongation of rice, caster bean, mung bean and other plants.[9,10] In order to extend and evaluate such a sporadic knowledge obtained from natural fields, and to know the relationship between structure and activities, the authors synthesized a number of isocoumarins and examined their biological activities on plants and fungi.

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MATERIALS AND METHODS

1. Synthesis

1.1 3–Acetoxy–4–acetylisocoumarins[11]

A mixture of a homophthalic acid (0.1 mole), a catalytic amount of pyridine and acetic anhydride (30 ml) was heated at about 50°C for 1 hr. When the reaction was over, the mixture was cooled and white precipitates obtained were collected by filtration. The precipitates were washed with diisopropyl ether and recrystallized from appropriate solvents to yield 3–acetoxy–4–acetylisocoumarins.

1.2 3–Methylisocoumarins[11]

2–Carboxyphenylacetones which are the hydrolysates of 3–acetoxy–4–acetylisocoumarins were recyclized by the following method (A) and (B).

A): A solution of 2–carboxyphenylacetone (1.5 g) in 100 ml of cyclizing reagent which was composed of $HClO_4$ (0.15 ml), acetic anhydride (10 ml) and ethyl acetate (90 ml)[12] was stirred at room temperature for 10 min. The solution was concentrated to one-third of its original volume *in vacuo* and washed with 5% sodium bicarbonate solution and then with water. The organic layer was dried over

anhydrous sodium sulfate and evaporated *in vacuo* to yield slight yellow solid. Recrystallization from diisopropyl ether gave 3–methylisocoumarin (1.1 g) as white crystals, mp 67–68°C.

B): Two grams of 2–carboxyphenylacetone was added to 50 ml of 80% sulfuric acid at 0°C. Stirring was continued for 8 hrs at 3–5°C. After the reaction, the reaction mixture was poured into 300 g of crushed ice and precipitated crystals were extracted with ether several times. The ether layer was dried over anhydrous sodium sulfate and evaporated *in vacuo* to yield white crystals, mp 67–68°C.

1.3 4–Ethoxycarbonyl–3–methylisocoumarins[13]

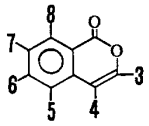
2–Halobenzoic acid (0.1 mole) and cuprous bromide (0.8 g) were suspended in 60 ml of dry ethyl acetoacetate. To this suspension, sodium hydride (8.7 g) was added under a dry N_2 atmosphere.

When the addition of sodium hydride had been completed, the mixture was continuously stirred at 80°C until an aliquot diluted with water became neutral. The aqueous layer was filtered and acidified with hydrochloric acid. Ethyl 2–(2–carboxyphenyl)acetoacetate was obtained as a dark oil in a good yield. The cyclization of ethyl 2–(2–carboxyphenyl)acetoacetate was pursued by the following methods, A′, B′ and C′.

A′): Ethyl 2–(2–carboxyphenyl)acetoacetate (2 g) in concentrated hydrochloric acid (20 ml) was heated at 80°C for 3 hrs. After cooling, the precipitated crystals were collected by filtration and recrystallized from diisopropyl ether to yield 4–ethoxycarbonyl–3–methylisocoumarin.

B′): Ethyl 2–(2–carboxyphenyl)acetoacetate (2 g) in dry benzene was refluxed for 5 hrs with a catalytic amount of *p*–toluenesulfonic

Table 1 Synthesized isocoumarin derivatives.



Compd. No.	Substituent at 3	4	5	6	7	8	Solvent for recrystn.[a]	Yield (%)	m.p. (°C)	Mol. formula[b]	Refr.
1	Me	*					D	85	67	$C_{10}H_8O_2$	
2	OAc	Ac					D	84	138	$C_{13}H_{10}O_5$	
3	Me			NO_2			D	53	183	$C_9H_5O_4N$	
4	Me				NO_2		D	25	140	$C_9H_5O_4N$	
5	Me			Me			D	84	62	$C_{11}H_{10}O_2$	
6	Me		Me				D	70	132	$C_{11}H_{10}O_2$	
7	Me				Me		D	76	132	$C_{11}H_{10}O_2$	
8	Me			OMe			D	83	98	$C_{11}H_{10}O_3$	
9	OAc	Ac		OMe			D	95	124	$C_{14}H_{12}O_6$	
10	OAc	Ac		OMe	OMe		D	75	210	$C_{15}H_{14}O_7$	
11	Me			OMe	OMe		D	70	120	$C_{12}H_{14}O_4$	
12	Me					OAc	D	34	111	$C_{12}H_{10}O_3$	
13	Me			Me	Me	OAc	D	70	197	$C_{14}H_{14}O_3$	
14	Me	COOEt		OMe	OMe		D	78	162	$C_{15}H_{16}O_6$	method C
15	Me	COOEt		Cl			D	72	135	$C_{13}H_{11}O_4Cl$	method C
16	Me	COOEt			Cl		D	71	102	$C_{13}H_{11}O_4Cl$	method C
17		COOH					E	17	175	$C_{10}H_6O_4$	$\nu_{C=O}$ 1665 cm^{-1}
18		COOH		Me			E	25	198	$C_{11}H_8O_4$	$\nu_{C=O}$ 1670
19		COOH		Cl			F	45	190	$C_{10}H_6O_4Cl$	$\nu_{C=O}$ 1757, 1680
20		COOH			Cl		F	48	198	$C_{10}H_6O_4Cl$	$\nu_{C=O}$ 1735, 1670

* Blank spaces are presenting H

a) Solvent for recrystallization

b) Analyses for C, H, and N, within ± 0.4% of calculated values except where noted.

D: Diisopropyl ether, E: Ethanol, F: aq. Ethanol

acid. When the reaction was over, the benzene layer was removed *in vacuo* and the residue was recrystallized from diisopropyl ether to yield 4–ethoxycarbonyl–3–methylisocoumarin in a good yield.

C'): Ethyl 2–(2–carboxyphenyl)acetoacetate (2 g) was mixed with sulfuric acid (85%, 50 ml) below 5°C and stirring was continued for 15 hrs below 5°C. When the reaction was over, the reaction mixture was poured into crushed ice and the precipitated crystals were collected by filtration, washed with water, and recrystallized from diisopropyl ether. Thus, 4–ethoxycarbonyl–3–methylisocoumarin was obtained in a good yield.

1.4 4–Carboxyisocoumarins[14]

In 15 ml of absolute methanol, 0.024 mole of metallic sodium was added. When the sodium was completely dissolved, 0.02 mole of dimethyl homophthalate was added at room temperature. After the addition of 0.03 mole of dry ethyl formate, the reaction mixture was continuously stirred for 12 hrs at room temperature. When the reaction was over, the reaction mixture was poured into 70 ml of water, acidified with dilute hydrochloric acid and extracted with ether several times. The ether layer was dried over anhydrous sodium sulfate and evaporated *in vacuo* to give brownish oily residue, which was dimethyl α–formylhomophthalate. To this residue, 5 ml of concentrated hydrochloric acid was added and the mixture was heated for 5 hrs on a boiling water bath. After cooling, the precipitated brown crystals were collected by filtration, washed with water and recrystallized from appropriate solvent.

The isocoumarin derivatives thus synthesized were enumerated in Table 1.

2. Bioassay

2.1 Test of phytotoxic activity

Examination of pre-emergent herbicidal activity was carried out on a manner described

Table 2 Effect of isocoumarin derivatives on plant growth (at 100 ppm) and *Asp. niger* (at 500 γ).

Compd. No.	Radish		Rice		Barnyard grass		*Asp. niger*
	Stem	Root	Stem	Root	Stem	Root	
1	+++	++	±	±			6 (mm)*
2	−−	−−	±	±			
3	±	−	±	+			
4	±	±	±	±			
5	±	−	±	−			12
6	−	−−	±	±			6
7	−−−	−−	±	±			6
8	−−	−−	±	+			13
9	±	±	±	±			
10	−	−−	±	±			
11	−−	−−	±	−−			
12	−	−−	+	−			
13	±	±	±	−			
14	×	×	±	±	±	±	
15	×	×	−	×	−	−−	
16	−−	−−−	±	−			
17	+	+	±	−−			
18	−	−−	±	−			
19	×	×	−−−	×	±	−−	
20	×	×	±	−−−	±	−	

Comp. No. is referring to the Table 1

+++, 80% or higher stimulation; ++, 50–80% stimulation; +, 20–50% stimulation; ±, 0–20% stimulation and 0–20% inhibition; −, 20–50% inhibition; −−, 50–80% inhibition; −−−, 80% or higher inhibition; ×, complete inhibition.

* Diameter of inhibitory circle.

previously.[15] In each Petri-dish, 20 seeds were placed and kept in a dark place at 25°C. The activity was evaluated after 5–7 days incubation inspecting the rate of growth inhibition on both stem and root. The results are showed in Table 2.

2.2 Test of fungicidal activity

Test for the fungicidal activity on *Asp. niger* was carried out in the similar manner described previously.[15]

RESULTS AND DISCUSSION

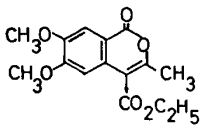
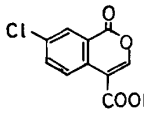
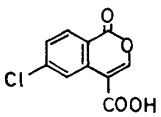
Phytotoxicity of isocoumarins on the germination and seedling growth of radish and rice is summarized in Table 2. A number of isocoumarins are phytotoxic, in particular, compounds No. 14, 15, 19 and 20 were highly phytotoxic to radish at 100 ppm. But only three isocoumarins 15, 19 and 20 showed inhibitory effect on the growth of rice seedling.

It is therefore thought that radish is more susceptible to isocoumarins than rice and barnyard grass. For four isocoumarins, No. 14, 15, 19 and 20 which inhibited the growth of radish completely at 100 ppm, the bioassay on rice, radish and barnyard grass was pursued at lower concentrations. The results were shown in Table 3 and Fig. 1, respectively.

The isocoumarin (No. 14) showed fairly strong plant growth regulating activity on radish but rice and barnyard grass were scarcely affected. The isocoumarins (No. 19 and 20) were the isomers each other. The former was herbicidally active to radish, rice and barnyard grass at 100 ppm and 50 ppm. But this compound stimulated the growth of radish and rice at lower concentrations, while barnyard grass was not stimulated. The latter (No. 20) inhibited to growth of stem and root of radish at 30 ppm to the extent of 47% and 54% respectively. But it considerably stimulated the elongation of radish root at lower concentrations. As for rice plant, the isocoumarin (No. 20) inhibited the elongation of stem and root to the extent of 13% and 67% respectively at 100 ppm.

But at 10 ppm, it stimulated the elongation to some extent. This compound also inhibited

Table 3 Plant growth regulating activity of isocoumarin derivatives.

Compound	Dose (ppm)	Growth rate (%)*					
		Radish		Rice		Barnyard Grass	
		Stem	Root	Stem	Root	Stem	Root
	100	0.0	0.0	105.3	105.1	103.2	111.3
	50	—	—	96.7	98.2	108.9	111.5
	30	37.0	9.2	—	—	—	—
	10	82.1	61.6	101.7	103.0	98.5	113.4
	3	106.7	172.6	—	—	—	—
	100	0.0	0.0	86.7	32.9	109.6	81.4
	50	—	—	89.7	41.2	107.4	87.0
	30	53.2	46.3	—	—	—	—
	10	99.1	159.5	108.7	127.9	—	—
	5	—	—	—	—	97.8	111.0
	1	119.4	131.0	—	—	—	—
	100	0.0	0.0	25.7	0.0	105.0	46.0
	50	25.0	10.3	78.0	36.8	102.3	64.7
	30	87.9	114.5	—	—	—	—
	10	91.8	155.2	93.7	93.1	104.2	106.2
	3	122.7	137.0	99.4	137.7	—	—
	1	125.6	125.6	101.6	117.3	—	—
	0.3	—	—	109.2	113.2	—	—
	0.1	—	—	101.8	107.5	—	—

—: Not tested

* : Growth rate $= \frac{\text{Length of the treated plants}}{\text{Length of the control plants}} \times 100$

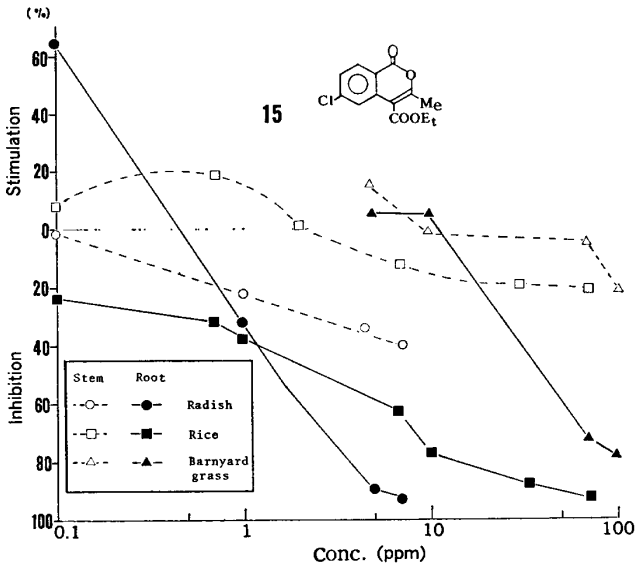


Fig. 1 Plant growth regulating activity of 4–ethoxycarbonyl–6–chloro–3–methylisocoumarin.

the differentiation and the growth of the adventitious roots in low concentrations and made the main root continue growing. Consequently, the length of main root of the treated rice at 10 ppm reached to 250%, compared with that of control rice.

Among the synthesized isocoumarins, No. 15 showed the most interesting activity to the plants. This compound strongly inhibited the elongation of rice root. The length of the treated root is about a half of control even at 1 ppm. Even at 0.1 ppm, the root elongation of rice was still inhibited to some extent, while the root of radish was elongated.

From these results, it seems that the isocoumarins, which possess carboxy or ethoxycarbonyl group at 4 position, have strong plant growth regulating activity.

On the other hand, only five compounds No. 1, 5, 6, 7 and 8 showed fungicidal activity on *Asp. niger*. 3,6–Dimethoxyisocoumarin (5) and 6–methoxy–3–methylisocoumarin (8) formed inhibitory circles (d=12 mm and 13 mm respectively), when 500 γ of the compounds were applied on the paper disk. Other 3–methylisocoumarins (1, 6 and 7) showed weak fungicidal activity, while 3–methylisocoumarins which possess carboxyl or ethoxycarbonyl substituent at 4–position, have no fungicidal activity.

From these results, it is assumed that 3–methyl group is required for the fungicidal activity and the substituent group at 6–position enhances the activity. But the carboxylate sustituents at 4–position extinguish the activity.

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要　　約

イソクマリン類の合成と生物活性

吉川博道，谷口栄二，前川一之

Homophthalic acids およびその合成中間体を用いて，3–acetoxy–4–acetylisocoumarins, 3–methylisocoumarins, 4–ethoxycarbonyl–3–methylisocoumarins, 4–carboxyisocoumarins の計 20 種の誘導体を合成した．得られた化合物を用い，大根，稲，ヒエ *Asp. niger* に対する作用を検討した結果，興味ある活性が見いだされた．すなわち，1) A 環に電子吸引基をもたない 3–methylisocoumarins が *Asp. niger* に対して，生育阻害作用をもつこと，2) 6 または 7 位が塩素置換された 4–carboxyisocoumarins が稲，大根に高濃度では生育を阻害するが，低濃度では生育促進的

に働くこと，3) 4–ethoxycarbonyl–3–methylisocoumarins が，4–carboxyisocoumarins と同様に，生長調整的に働くことを見いだした．とくに 4–ethoxycarbonyl–6–chloro–3–methylisocoumarin は 0.1 ppm の低濃度においても，稲の根の伸長を阻害した．また，7–chloro–4–carboxyisocoumarin は 10 ppm で，稲の不定根の分化を阻害し，主根の伸長を促進するという，ジベレリン様活性を示した．