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Structure-Activity Relationships in Tetronic Acids and Their Copper(II) Complexes¹⁾

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3-Acyltetronic acids and their copper complexes, which possess a tricarbonylmethane structure, were prepared and tested for antimicrobial activity. 3-(1-Iminoalkyl)tetronic acids and their copper complexes were prepared and tested for inhibitory activity towards chlorophyll development of plants. It was found that the 3-decanoyl group was essential for the appearance of the former activity and the 3-(1-iminoethyl) group for that of the latter.

Keywords—tricarbonylmethane structure; 3-acyltetronic acids; 3-(1-iminoalkyl)-tetronic acids; copper(II) complexes; antimicrobial activity; chlorosis

Tetracycline, usnic acid and dehydroacetic acid, which are typical antimicrobial agents, all possess a tricarbonylmethane structure. In addition, Ukita and his co-workers reported that 3-decanoyl-4-hydroxycoumarin exhibited the most powerful antibacterial activity against *Staphylococcus aureus* among various 3-acyl-4-hydroxycoumarin derivatives.³⁾

We therefore selected some 3-acyltetronic acids as model compounds to investigate the structure-activity relationships involved in the antimicrobial activity, because these compounds have the tricarbonylmethane structure, and can form metal complexes, which should increase the liposolubility and the permeability through cell walls.

It is also known that 3-(1-iminoethyl)-5-methyltetronic acid inhibits the chlorophyll development of plants.⁴⁾ Therefore, imino derivatives of 3-acyltetronic acids were also synthesized and tested for inhibitory activity.

3-Acyltetronic acids were prepared by applying Mulholland's method,⁵⁾ followed by acylation in the presence of titanium tetrachloride,⁶⁾ boron trifluoride etherate⁷⁾ or aluminum chloride as shown in Chart 1, yielding 3-acetyl (**5a**, **5b**), 3-hexanoyl (**6a**, **6b**), 3-decanoyl (**7a**, **7b**) and 3-phenylacetyl (**8a**, **8b**) derivatives, respectively.

3-(1-Iminoalkyl)tetronic acid derivatives, such as the 3-(1-iminoethyl) (**9a**, **9b**), 3-(1-imino-hexyl) (**10a**, **10b**), 3-(1-iminodecyl) (**11a**, **11b**) and 3-(1-imino-2-phenylethyl) (**12b**) derivatives, were synthesized by treatment of the corresponding 3-acyl derivatives (**5a**, **5b**, **6a**, **6b**, **7a**, **7b**, **8b**) with methanolic ammonia at 100° in a sealed tube.

The structures of the newly obtained compounds, 3-acyltetronic acids (**6a**, **6b**, **7a**, **7b**, **8a**, **8b**) and their imino derivatives (**9a**, **10a**, **10b**, **11a**, **11b**, **12b**), were confirmed by elemental analyses, and infrared (IR) and nuclear magnetic resonance (NMR) spectral data.

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Chart 1

Copper complexes were prepared by treatment of 3-acyl and 3-(1-iminoalkyl)tetrionic acids with copper(II) acetate in warm aqueous ethanol.

The elemental analyses indicated that these complexes contained copper(II) and ligand in a molar ratio of 1:2. On the other hand, from magnetic susceptibility measurements at

TABLE I. Copper(II) Complexes of 3-Acyl and 3-(1-Iminoalkyl)tetrionic Acids

Compound	Appearance	mp (°C)	Formula	Analysis (%)			μ_{eff} (B.M.)
				Found	Calcd.		
				C	H	N	
5a-Cu	Green cryst. powder	>300	C ₁₂ H ₁₀ CuO ₈	41.54 (41.68)	2.88 2.92	— (—)	1.89
5b-Cu	Green prisms	>300	C ₁₄ H ₁₄ CuO ₈	44.89 (44.98)	3.75 3.77	— (—)	—
6a-Cu	Blue cryst. powder	229—230	C ₂₀ H ₂₆ CuO ₈	52.33 (52.44)	5.74 5.73	— (—)	1.96
6b-Cu	Blue prisms	195.5	C ₂₂ H ₃₀ CuO ₈	54.32 (54.37)	6.28 6.22	— (—)	2.06
7a-Cu	Blue cryst. powder	220.5—223	C ₂₈ H ₄₂ CuO ₈	58.72 (58.97)	7.52 7.44	— (—)	1.92
7b-Cu	Blue needles	185.5—188.5	C ₃₀ H ₄₆ CuO ₈	60.16 (60.23)	8.08 7.75	— (—)	1.92
8b-Cu	Green prisms	145	C ₂₆ H ₂₂ CuO ₈ ·2H ₂ O	55.63 (55.56)	4.69 4.66	— (—)	—
9a-Cu	Violet cryst. powder	>300	C ₁₂ H ₁₂ CuN ₂ O ₆	41.81 (41.92)	3.63 3.53	7.89 8.15	1.82
9b-Cu	Violet cryst. powder	290	C ₁₄ H ₁₆ CuN ₂ O ₆	45.21 (45.22)	4.40 4.30	7.42 7.53	1.85
10a-Cu	Violet plates	173—174	C ₂₀ H ₂₈ CuN ₂ O ₆	52.61 (52.67)	6.24 6.29	5.95 6.14	1.88
10b-Cu	Violet prisms	175.5	C ₂₂ H ₃₂ CuN ₂ O ₆	54.71 (54.59)	6.82 6.67	5.70 5.79	—
11a-Cu	Blue prisms	135.5—137	C ₂₈ H ₄₄ CuN ₂ O ₆	59.11 (59.18)	8.00 7.80	4.87 4.93	1.87
11b-Cu	Blue needles	143—145	C ₃₀ H ₄₈ CuN ₂ O ₆ ·1/2H ₂ O	59.53 (59.53)	8.30 8.16	4.53 4.63	—
12b-Cu	Violet prisms	>300	C ₂₆ H ₂₄ CuN ₂ O ₆ ·1/2H ₂ O	58.95 (58.58)	4.71 4.73	5.18 5.17	1.92

room temperature using the Gouy method, the effective magnetic moments, μ_{eff} , were calculated to be within 1.8—2.0 B.M. (Table I). These results suggested that these complexes have the structure shown in Chart 2.

Chart 2

The results of the antimicrobial activity tests on the 3-acyl and 3-(1-iminoalkyl)tetronic acids and their copper complexes are summarized in Table II. Some of these compounds inhibited the growth of *Bacillus subtilis* (IFO-3513) and *Staphylococcus aureus* (IFO-3061), but not that of *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens* or molds.

In summary, 3-acetyltetronic acids (**5a**, **5b**), 3-hexanoyltetronic acids (**6a**, **6b**) and 3-phenylacetyltetronic acid (**8b**) do not show antimicrobial activities, but 3-decanoyltetronic acids (**7a**, **7b**) do show strong activity. Conversion of **7a** and **7b** to their copper complexes, **7a-Cu** and **7b-Cu**, does not affect the activity, while conversion to the 3-(1-iminodecyl)tetronic acid derivatives (**11a**, **11b**) results in complete loss of the activity.

TABLE II. Antimicrobial Activities of Tetronic Acids and Their Copper(II) Complexes (MIC: mcg/ml)

Microorganism	Compound						
	5a 5a-Cu	5b 5b-Cu	6a 6a-Cu	6b 6b-Cu	7a	7a-Cu	7b
<i>B. subtilis</i> (IFO-3513)	>100	>100	>100	>100	6.25	12.5	12.5
<i>St. aureus</i> (IFO-3061)	>100	>100	>100	>100	6.25	12.5	3.12

Microorganism	Compound						
	7b-Cu	8b 8b-Cu	9b 9b-Cu	10b 10b-Cu	11a 11a-Cu	11b 11b-Cu	12b 12b-Cu
<i>B. subtilis</i> (IFO-3513)	12.5	>100	>100	>100	>100	>100	>100
<i>St. aureus</i> (IFO-3061)	6.25	>100	>100	>100	>100	>100	>100

The effects of 3-(1-iminoalkyl)tetronic acid derivatives on chlorophyll development of rice and radish are shown in Table III. Although 3-(1-iminoethyl)-5-methyltetronic acid (**9b**) causes distinct chlorosis, as reported in the literature,⁴⁾ 3-(1-iminoethyl)tetronic acid (**9a**) is less active than **9b**. In the case of their copper(II) complexes (**9a-Cu**, **9b-Cu**), rather weak chlorosis and growth inhibition of shoots and roots were observed. On the other hand,

3-(1-iminoethyl)tetronic acid (**10a**), 3-(1-iminodecyl)-5-methyltetronic acid (**11b**) and their copper(II) complexes (**10a**-Cu, **11b**-Cu) do not cause chlorosis.

TABLE III. Degree of Chlorosis and Growth Inhibition^{a)}

Compound	Concentration	Radish			Rice		
		Degree of chlorosis	Growth inhibition		Degree of chlorosis	Growth inhibition	
			Shoots	Roots		Shoots	Roots
9a	100	±	—	—	—	nt	nt
9a -Cu	100	+	—	++	—	++	+++
9b	10	±	—	—	+	—	nt
9b	100	+++	—	—	++	±	±
9b -Cu	100	+	—	+	nt	nt	nt
10a	100	—	—	—	—	—	—
10a -Cu	100	—	—	—	—	++	+++
11b	100	—	—	—	nt	nt	nt
11b -Cu	100	—	—	—	nt	nt	nt

a) Negative (—); >25%(+); >50%(++); >75%(+++); 100%(++++); not tested (nt).

It may be concluded, therefore, that the structure of the acyl substituent at the 3-position is important for the appearance of both activities. The antimicrobial activity requires a 3-decanoyl group and the inhibitory activity on chlorophyll development requires a 3-(1-iminoethyl) group in the molecule. In the latter case, the substituent at the 5-position affects the activity.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus, model MP-S3, and are uncorrected. Infrared (IR) spectra were measured in Nujol mulls with a Hitachi EPI-S infrared spectrometer and nuclear magnetic resonance (NMR) spectra were measured with a Hitachi Perkin-Elmer R-20A spectrometer at 60 MHz using tetramethyl silane (TMS) as an internal standard. The magnetic susceptibility was determined at room temperature by means of a Gouy magnetic apparatus with a Mettler H51AR microbalance and a Tokyo Giken WM-III electromagnet in a field of about 9000 G.

Tetronic Acid (4a)—Ethyl 2-ethoxy-4,5-dihydro-4-oxofuran-3-carboxylate (**3a**)⁴⁾ (71.3 g) was left to stand in 2.25 N aqueous KOH solution (1096 ml) for 5 days at room temperature. The solution was acidified with conc. HCl with ice-cooling. The resulting acidic solution was salted out with NaCl and extracted with ethyl acetate. The organic layer was dried over Na₂SO₄ and evaporated down. The residue was recrystallized from ethyl acetate, yielding **4a** (16 g, 62.5%) as colorless needles. mp 146° (lit.⁴⁾ 138–140°. IR $\nu_{\text{max}}^{\text{Nujol}}$ cm⁻¹: 2630 (OH), 1680 (C=O), 1635 (C=C).

5-Methyltetronic Acid (4b)—Using the same procedure as for **4a**, ethyl 2-ethoxy-4,5-dihydro-5-methyl-4-oxofuran-3-carboxylate (**3b**)⁴⁾ (12.1 g) was hydrolyzed, decarboxylated and recrystallized from ethyl acetate and petroleum ether to give **4b** (4.8 g, 74%) as colorless prisms. mp 122–122.5° (lit.⁴⁾ 118–120°. IR $\nu_{\text{max}}^{\text{Nujol}}$ cm⁻¹: 2650, 2550 (OH), 1712 (C=O), 1629 (C=C).

Acylation of 4a and 4b—Method 1: Acyl chloride was added to a solution of **4a** or **4b** in nitrobenzene and then titanium tetrachloride was added dropwise with stirring and cooling (–10°). The mixture was heated at 85° for 4 hr. After adding 5 N HCl to the cooled reaction mixture, the organic layer was separated and extracted with 10% aqueous NaHCO₃ solution. The alkaline solution was acidified with conc. HCl and extracted with chloroform. The chloroform solution was dried over Na₂SO₄ and evaporated down *in vacuo*. The residue was recrystallized from ethyl ether and petroleum ether.

Method 2: Acyl chloride was added a solution of **4a** or **4b** in dioxane and then boron trifluoride etherate was added dropwise with stirring at 16°. The mixture was refluxed for 4 hr. After cooling, water was added to the reaction product and the mixture was extracted three times with chloroform. 3-Acyltetronic acids were isolated from the chloroform solution and recrystallized by the procedure given in method 1.

Method 3: **4a** or **4b** was dissolved in nitrobenzene and mixed with acyl chloride. The solution was heated at 80° for 4 hr in the presence of anhydrous aluminum chloride. Water was added to the reaction product with ice-cooling and then the mixture was extracted with ethyl ether. 3-Acyltetronic acids were isolated from the ether solution and purified by the procedure given in method 1.

3-Acetyltetronic Acid (5a)—5a (0.37 g) was obtained from 4a (1 g) and acetyl chloride by method 1 as colorless needles. mp 81–83° (lit.⁸) 79°. IR $\nu_{\max}^{\text{CHCl}_3}$ cm⁻¹: 1780, 1700, 1670 (C=O), 1605 (C=C).

3-Acetyl-5-methyltetronic Acid (5b)—5b (0.94 g) was obtained from 4b (2.28 g) and acetyl chloride by method 1 as colorless prisms. mp 54–55° (lit.⁹) 54–55°. IR $\nu_{\max}^{\text{CHCl}_3}$ cm⁻¹: 1765, 1690, 1660 (C=O), 1610 (C=C).

3-*n*-Hexanoyltetronic Acid (6a)—6a (1.2 g) was obtained from 4a (1.5 g) and *n*-hexanoyl chloride by method 1 as colorless needles. mp 72–74°. IR $\nu_{\max}^{\text{CHCl}_3}$ cm⁻¹: 1775, 1690, 1665 (C=O), 1600 (C=C). NMR (CDCl₃) δ : 0.92 (3H, bt, $J=6$ Hz, CH₂CH₃), 1.0–2.0 (6H, m, (CH₂)₃CH₃), 2.93 (2H, t, $J=7$ Hz, COCH₂), 4.6 (2H, d, $J=7$ Hz, CH₂O), 12.07 (1H, s, OH). Anal. Calcd. for C₁₀H₁₄O₄: C, 60.59; H, 7.13. Found: C, 60.71; H, 7.27.

3-*n*-Hexanoyl-5-methyltetronic Acid (6b)—6b (0.66 g) was obtained from 4b (2 g) and *n*-hexanoyl chloride by method 3 as colorless prisms. mp <30°. IR $\nu_{\max}^{\text{CHCl}_3}$ cm⁻¹: 1773, 1658 (C=O), 1603 (C=C). NMR (CDCl₃) δ : 0.9 (3H, bt, $J=6$ Hz, CH₂CH₃), 1.1–2.0 (6H, m, (CH₂)₃CH₃), 1.52 (3H, d, $J=7$ Hz, CHCH₃), 2.92 (2H, t, $J=7$ Hz, COCH₂), 4.81 (1H, q, $J=7$ Hz, CHCH₃), 11.7 (1H, s, OH).

3-*n*-Decanoyltetronic Acid (7a)—7a (2.18 g) was obtained from 4a (2 g) and *n*-decanoyl chloride by method 2 as colorless prisms. mp 74–74.5°. IR $\nu_{\max}^{\text{CHCl}_3}$ cm⁻¹: 1775, 1693, 1670 (C=O), 1600 (C=C). NMR (CDCl₃) δ : 0.89 (3H, bt, $J=4$ Hz, CH₂CH₃), 1.1–2.0 (14H, m, (CH₂)₇CH₃), 2.95 (2H, t, $J=7$ Hz, COCH₂), 4.62 (2H, bs, CH₂O), 11.1 (1H, s, OH). Anal. Calcd. for C₁₄H₂₂O₄: C, 66.11; H, 8.72. Found: C, 66.50; H, 8.69.

3-*n*-Decanoyl-5-methyltetronic Acid (7b)—7b (3.77 g) was obtained from 4b (2 g) and *n*-decanoyl chloride by method 2 as colorless needles. mp 44.5–46°. IR $\nu_{\max}^{\text{CHCl}_3}$ cm⁻¹: 1768, 1690, 1662 (C=O), 1603 (C=C). NMR (CDCl₃) δ : 0.89 (3H, bt, $J=6$ Hz, CH₂CH₃), 1.1–2.0 (14H, m, (CH₂)₇CH₃), 1.53 (3H, d, $J=7$ Hz, CHCH₃), 2.93 (2H, t, $J=7$ Hz, COCH₂), 4.81 (1H, q, $J=7$ Hz, CHCH₃). Anal. Calcd. for C₁₅H₂₄O₄: C, 67.17; H, 9.01. Found: C, 67.10; H, 9.17.

3-Phenylacetyltetronic Acid (8a)—8a (0.44 g) was obtained from 4a (1 g) and phenylacetyl chloride by method 3 as colorless prisms. mp 73–77°. IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3100 (OH), 1755, 1650 (C=O), 1605 (C=C). NMR (CDCl₃) δ : 4.18 (2H, s, CH₂C₆H₅), 4.6 (2H, s, CH₂O), 7.29 (5H, s, C₆H₅), 11.91 (1H, s, OH).

3-Phenylacetyl-5-methyltetronic Acid (8b)—8b (9 g) was obtained from 4b (10 g) and phenylacetyl chloride by method 3 as colorless prisms. mp 73–76°. IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3150 (OH), 1751, 1662 (C=O), 1609 (C=C). NMR (CDCl₃) δ : 1.52 (3H, d, $J=7$ Hz, CHCH₃), 4.20 (2H, s, CH₂C₆H₅), 4.69 (1H, q, $J=7$ Hz, CHCH₃), 7.3 (5H, s, C₆H₅), 10.6 (1H, s, OH). Anal. Calcd. for C₁₃H₁₂O₄: C, 67.24; H, 5.21. Found: C, 67.41; H, 5.14.

Imination of 3-Acyltetronic Acids—General Method: 3-Acyltetronic acid and a 25% methanolic solution of ammonia were heated together at 100° in a sealed tube for 3 hr. The reaction mixture was concentrated *in vacuo* to give a solid residue, which was recrystallized from a mixture of methanol and ethyl ether.

3-(1-Iminoethyl)tetronic Acid (9a)—9a (0.2 g) was obtained from 5a (0.22 g) by the general method as colorless needles. mp 236–239°. IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3325, 3170 (NH, OH), 1725 (C=O), 1648 (C=N). NMR (DMSO-*d*₆) δ : 2.38 (3H, s, CH₃), 3.32 (1H, s, NH), 4.35 (2H, s, CH₂O), 9.55 (1H, bs, OH). Anal. Calcd. for C₆H₇NO₃: C, 51.05; H, 5.01; N, 9.93. Found: C, 50.87; H, 4.92; N, 9.86.

3-(1-Iminoethyl)-5-methyltetronic Acid (9b)—9b (0.3 g) was obtained from 5b (0.5 g) by the general method as a colorless crystalline powder. mp 162° (lit.⁷) 159–160°. IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3230, 3060 (NH, OH), 1705 (C=O), 1650 (C=N), 1610 (C=C). Anal. Calcd. for C₇H₉NO₃: C, 54.19; H, 5.84; N, 9.03. Found: C, 54.11; H, 5.74; N, 9.28.

3-(1-Iminohexyl)tetronic Acid (10a)—10a (0.49 g) was obtained from 6a (0.5 g) by the general method as colorless needles. mp 114–115°. IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3270, 3150 (NH, OH), 1700 (C=O), 1650 (C=N). NMR (DMSO-*d*₆) δ : 0.88 (3H, bt, $J=6$ Hz, CH₃), 1.05–1.7 (6H, m, (CH₂)₃CH₃), 2.75 (2H, bt, $J=7$ Hz, C(=NH)-CH₂), 3.32 (1H, s, NH), 4.35 (2H, s, CH₂O), 9.5 (1H, bs, OH). Anal. Calcd. for C₁₀H₁₅NO₃: C, 60.88; H, 7.68; N, 7.10. Found: C, 60.64; H, 7.62; N, 7.14.

3-(1-Iminohexyl)-5-methyltetronic Acid (10b)—10b (0.54 g) was obtained from 6b (1 g) by the general method as colorless prisms. mp 83°. IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3300, 3160 (NH, OH), 1710 (C=O), 1644 (C=N). NMR (CDCl₃) δ : 0.91 (3H, bt, $J=6$ Hz, CH₂CH₃), 1.1–2.0 (6H, m, (CH₂)₃CH₃), 1.45 (3H, d, $J=7$ Hz, CHCH₃), 2.9 (2H, bt, $J=8$ Hz, C(=NH)CH₂), 4.56 (1H, q, $J=7$ Hz, CHCH₃), 7.74 (1H, s, NH), 9.35–10.4 (1H, m, OH).

3-(1-Iminodecyl)tetronic Acid (11a)—11a (0.49 g) was obtained from 7a (0.5 g) by the general method as light yellow needles. mp 120–122°. IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3270, 3130 (NH, OH), 1698 (C=O), 1653 (C=N). NMR (DMSO-*d*₆) δ : 0.88 (3H, bt, $J=6$ Hz, CH₂CH₃), 1.28 (14H, bs, (CH₂)₇CH₃), 2.65 (2H, bt, $J=7$ Hz, C(=NH)CH₂), 3.32 (1H, s, NH), 4.38 (2H, s, CH₂O), 9.5 (1H, bs, OH). Anal. Calcd. for C₁₄H₂₃NO₃: N, 5.53. Found: N, 5.55.

3-(1-Iminodecyl)-5-methyltetronic Acid (11b)—11b (3.4 g) was obtained from 7b (3.8 g) by the general method as colorless needles. mp 87–89°. IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3250 (NH), 1703 (C=O), 1650 (C=N). NMR (CDCl₃) δ : 0.89 (3H, bt, $J=4$ Hz, CH₂CH₃), 1.30 (14H, bs, (CH₂)₇CH₃), 1.45 (3H, d, $J=7$ Hz, CHCH₃), 2.93 (2H,

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m, C(=NH)CH₂, 4.55 (1H, q, $J=7$ Hz, CHCH₃), 6.80 (1H, bs, NH). *Anal.* Calcd. for C₁₅H₂₅NO₃: C, 67.38; H, 9.43; N, 5.24. Found: C, 67.23; H, 9.53; N, 5.54.

3-(1-Imino-2-phenylethyl)-5-methyltetronic Acid (12b)—**12b** (0.17 g) was obtained from **8b** (1 g) by the general method as colorless prisms. mp 125–126°. IR $\nu_{\text{max}}^{\text{Nujol}}$ cm⁻¹: 3260, 3120 (NH, OH), 1710 (C=O), 1654 (C=N). NMR (CDCl₃) δ : 1.45 (3H, d, $J=7$ Hz, CHCH₃), 4.32 (2H, d, $J=3$ Hz, CH₂C₆H₅), 4.56 (1H, q, $J=7$ Hz, CHCH₃), 6.6–7.1 (1H, bs, NH), 7.4 (5H, s, C₆H₅), 9.1–10.6 (1H, bs, OH).

Copper(II) Complexes of 3-Acyl and 3-(1-Iminoalkyl)tetronic Acids—General Method: A tetronic acid derivative was dissolved in ethanol and mixed with a solution of cupric acetate in aqueous ethanol (1:1) at 40°. The copper(II) complex, deposited after cooling, was recrystallized from a mixture of ethanol and water or DMSO and water. Physical data for the products are listed in Table I.

Antimicrobial Test—The minimum inhibitory concentration (MIC, mcg/ml) was measured as follows; bouillon agar (9 ml) was mixed with 1 ml of an aqueous solution containing a test compound dissolved by the addition of dimethyl formamide (DMF) and acetone to give various concentrations. The agar was then poured into a Petri dish. After solidification, the agar was streaked with test organism suspension and incubated at 33° for 18–20 hr. The MIC for each compound was defined as the lowest concentration inhibiting the growth of the test organisms.

Inhibition Tests for Chlorophyll Development and Plant Growth—The test compound was suspended in aqueous solution containing Tween 20 and acetone to give various concentrations (10, 100 mcg/ml). The solution (5 ml) was poured onto a Toyo filter paper (No. 2) in a Petri dish (diameter, 9 cm). Twenty radish seeds were put on the filter paper and cultured under irradiation with daylight fluorescent lamps (2000 lux) at 25° for 5 days. The solution (2 ml) was also poured into a glass tube (diameter, 3 cm; length, 12 cm) containing 10 rice seeds (strain: Nihonbare) and cultured under the same lighting conditions at 30° for 7 days.

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