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Structure-Activity Relationships in Tetramic Acids and Their Copper (II) Complexes[1]

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3-Acetyl and 3-decanoyltetramic acids with various substituents at the 5-position and their copper complexes, which possess a tricarbonylmethane structure, were prepared and tested for antimicrobial activity. 3-Decanoyltetramic acid derivatives and their copper complexes inhibited the growth of *Bacillus subtilis* and *Staphylococcus aureus*, but did not inhibited the growth of gram-negative bacteria and molds. 3-(1-Iminoethyl)-tetramic acids and their copper complexes were synthesized and tested for inhibitory activity towards chlorophyll development in plants.

Keywords——tricarbonylmethane structure; 3-acetyltetramic acids; 3-decanoyltetramic acids; 3-(1-iminoethyl)tetramic acids; copper(II) complexes; antimicrobial activity

In the previous paper,[3] we reported the synthesis of 3-acyltetronic acids and their copper (II) complexes, which possess a tricarbonylmethane structure. Tests of their antimicrobial activity indicated that the structure of the acyl substituent at the 3-position was important and that it must be a 3-decanoyl group for the appearance of activity against *Bacillus subtilis* (IFO-3513) and *Staphylococcus aureus* (IFO-3061).

On the other hand, tenuazoic acid,[4] streptolydigin,[5] tirandamycin[6] and malonomycin,[7] which are typical antibiotics, all possess a 3-acyltetramic acid moiety as the tricarbonylmethane structure.

We therefore synthesized 3-acetyl and 3-decanoyltetramic acids with various substituents at the 5-position and their copper (II) complexes, which should show increased liposolubility and permeability through cell walls,[8] and tested the antimicrobial activity of these compounds to investigate the structure-activity relationships.

It is also known that 3-(1-iminoethyl)-5-methyltetronic acid inhibits the chlorophyll development of plants.[3,9] Therefore, the imino derivatives of the 3-acetyltetramic acids were also synthesized and tested for inhibitory activity in order to investigate the correlation between tetronic acid and tetramic acid.

There are two routes for the preparation of the 3-acyltetramic acid derivatives. The most straightforward route involves acylating an enolate of 5-substituted tetramic acid (**1**).[10,11]

1) This work was presented at the Annual Meeting of the Kinki Branch, Pharmaceutical Society of Japan, Nishinomiya, Nov. 1978 and Kyoto, Nov. 1979.
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3) K. Tanaka, K. Matsuo, Y. Nakaizumi, Y. Morioka, Y. Takashita, Y. Tachibana, Y. Sawamura, and S. Kohda, *Chem. Pharm. Bull.*, **27**, 1901 (1979).
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9) C.L. Hamner and H.B. Tukey, *Botanical Gazz.*, **112**, 525 (1951).
10) R.C.F. Jones and S. Sumaria, *Tetrahedron Lett.*, **1978**, 3137.
11) V.J. Lee, A.R. Branfman, T.R. Herrin, and K.L. Rinehart, Jr., *J. Am. Chem. Soc.*, **100**, 4225 (1978).

A second attractive route involves the condensation of a β-keto carboxylic acid (or its equivalent) with an amine (α-amino acid ester) or the reaction of an aldehyde (or its equivalent) with N-(α-haloacetyl)amino acid ester in the presence of Zn (Reformatsky reaction) followed by oxidation to form a β-keto amide, and subsequent intramolecular Claisen condensation.[11,12]

For the preparation of the 3-acetyltetramic acid derivatives, the latter method was employed. Thus, the 3-acetyltetramic acids (**3**—**7**) were prepared by acetoacetylation of the amino acid esters (**2**) with diketene followed by cyclization with sodium methoxide in good yield.[12]

Both routes for the synthesis of the 3-decanoyltetramic acid derivatives were examined in the present study.

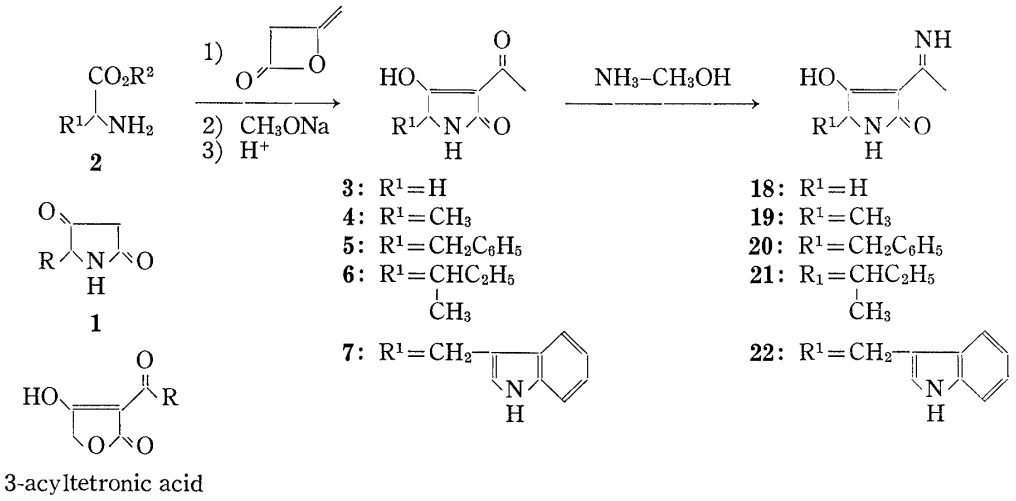


Chart 1

Jones *et al.*[10] reported the synthesis of 3-acetyl, 3-heptanoyl, 3-but-2-enoyl and 3-hexa-2,4-dienoyl-5-benzyltetramic acids by acylation of 5-benzyltetramic acid (**1**: R=$CH_2C_6H_5$) with the appropriate acyl chloride in the presence of Lewis acids, such as boron trifluoride or titanium tetrachloride.

According to Jones' method, decanoylation was attempted by reacting decanoyl chloride with 5-benzyltetramic acid (**1**: R=$CH_2C_6H_5$) in nitrobenzene or dioxane in the presence of titanium tetrachloride or boron trifluoride etherate as a catalyst. However, no positive spot appeared with ferric chloride spray reagent among the worked-up crude products.

We therefore turned our attention to the second route. Firstly, ethyl phenylalaninate was treated with bromoacetyl bromide to form ethyl N-bromoacetylphenylalaninate (**8**). The bromide (**8**) was reacted with decanal in a mixture of tetrahydrofuran and acetic acid in the presence of Zn–Cu couples[13] to give β-hydroxy amide (**9**), which was then oxidized with Jones' reagent to produce the β-keto amide (**10**) (18% yield from **8**) without purification, because of contamination with a reduced by-product, ethyl N-acetylphenylalaninate (**11**), which was difficult to remove. The β-keto amide (**10**) was then cyclized by the action of sodium methoxide in a mixture of methanol and benzene to furnish 5-benzyl-3-decanoyltetramic acid (**16**) in 54.5% yield.

Because of the relatively low yield, another method for the preparation of 3-decanoyltetramic acid derivatives was sought; the resulting reaction sequences are shown in Chart 3. Thus, ethyl glycinate (**2**) (R^1=H, R^2=C_2H_5) was acylated with 3-oxododecanoyl chloride (**12**) to form the β-keto amide (**13**) (R^1=H, R^2=C_2H_5) in 55.3% yield; this was treated with sodium methoxide as described above to form 3-decanoyltetramic acid (**14**) in 87.6% yield.

12) R.N. Lacy, *J. Chem. Soc.*, **1954**, 850.
13) E. Santaniello and A. Manzocchi, *Synthesis*, **1977**, 698.

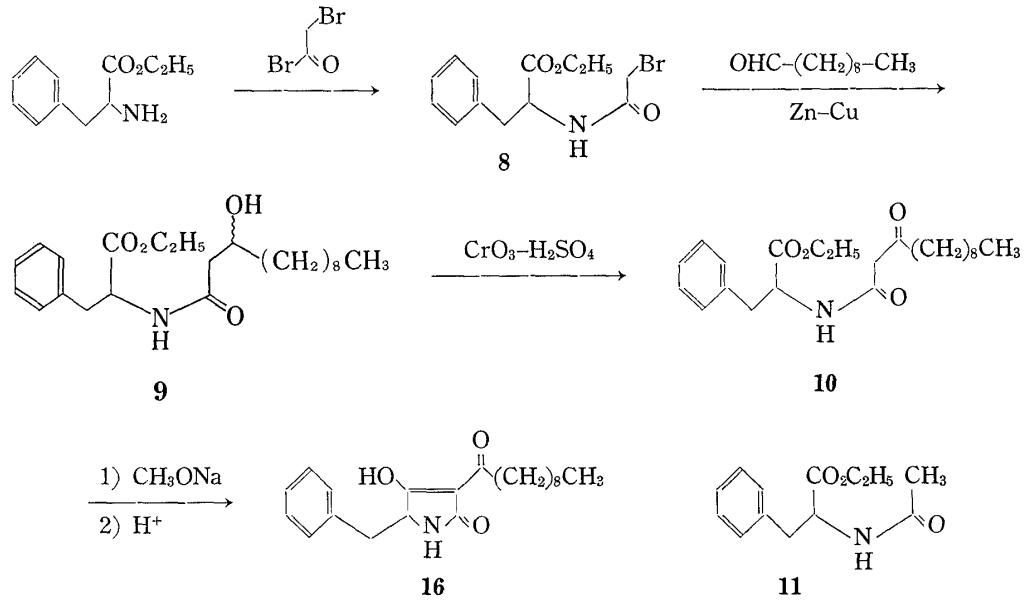


Chart 2

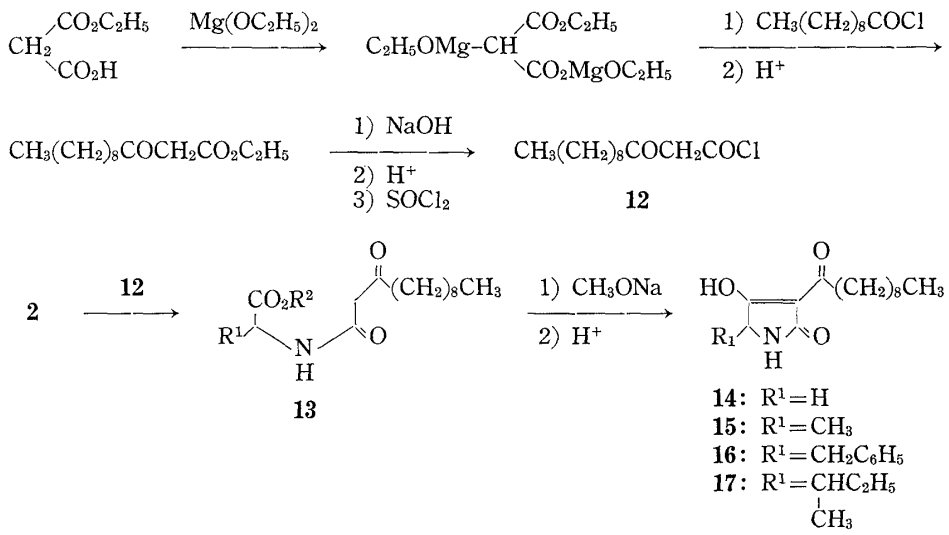


Chart 3

3-Oxododecanoyl chloride (**12**) was prepared by condensation of decanoyl chloride with the bis(ethoxymagnesio)enolate of monoethyl malonate, followed by decarboxylation, hydrolysis and chlorination with thionyl chloride. Other 3-decanoyltetramic acids, 5-methyl (**15**), 5-benzyl (**16**) and 5-(1-methylpropyl) (**17**) derivatives, were also synthesized by applying this latter method.

3-(1-Iminoethyl)tetramic acid derivatives (**18**—**22**) were prepared by treatment of the corresponding 3-acetyl derivatives (**3**—**7**) with methanolic ammonia at 100° in a sealed tube[3] (Chart 1).

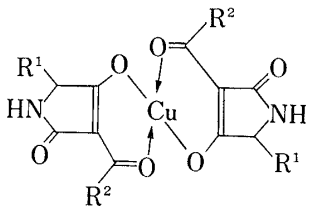
The structures of the newly obtained compounds, 3-acyltetramic acids (**7** and **14**—**17**) and their imino derivatives (**18**—**22**), were confirmed by elemental analyses and infrared (IR) and nuclear magnetic resonance (NMR) spectral data.

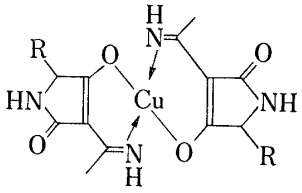
Copper complexes were synthesized by treatment of 3-acyl and 3-(1-iminoethyl)tetramic acid derivatives with copper (II) acetate in warm aqueous ethanol.[3]

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

TABLE I. Copper (II) Complexes of 3-Acyl and 3-(1-Iminoethyl)tetramic Acids

Compound	Appearance	mp (°C)	Formula	Analysis (%) Found (Calcd) C	H	N	μ_{eff} (B.M.)
3-Cu	Green cryst. powder	260(dec.)	$C_{12}H_{12}CuN_2O_6 \cdot 1/2H_2O$	38.59 (38.87	3.89 4.08	7.19 7.55)	—
4-Cu	Blue needles	>460	$C_{14}H_{16}CuN_2O_6 \cdot 1/2H_2O$	44.39 (44.15	4.47 4.50	7.37 7.36)	—
5-Cu	Green cryst. powder	177—181	$C_{26}H_{24}CuN_2O_6 \cdot H_2O$	57.37 (57.60	4.59 4.84	5.41 5.17)	—
6-Cu	Green cryst. powder	168—175	$C_{20}H_{28}CuN_2O_6 \cdot H_2O$	50.69 (50.67	6.24 6.40	5.85 5.91)	1.97
7-Cu	Green cryst. powder	208—213	$C_{30}H_{26}CuN_4O_6 \cdot H_2O$	58.13 (58.10	4.57 4.55	8.97 9.02)	1.75
14-Cu	Blue cryst. powder	266—268.5	$C_{28}H_{44}CuN_2O_6$	58.92 (59.17	7.91 7.82	5.11 4.93)	1.88
15-Cu	Blue cryst. powder	225—235	$C_{30}H_{48}CuN_2O_6 \cdot 1/2H_2O$	59.58 (59.52	7.95 8.18	4.64 4.63)	1.91
16-Cu	Blue cryst. powder	200—206	$C_{42}H_{56}CuN_2O_6$	67.33 (67.39	7.54 7.56	3.89 3.74)	1.86
17-Cu	Gray cryst. powder	201—205	$C_{36}H_{60}CuN_2O_6$	63.64 (63.53	8.70 8.91	4.18 4.12)	1.86
18-Cu	Purple cryst. powder	>300	$C_{12}H_{14}N_4O_4 \cdot 1/2H_2O$	40.84 (41.08	4.38 4.61	15.96 15.97)	—
19-Cu	Blue prisms	290(dec.)	$C_{14}H_{18}CuN_4O_4 \cdot 1/2H_2O$	44.59 (44.38	5.02 5.06	14.55 14.79)	—
20-Cu	Gray prisms	>300	$C_{26}H_{26}CuN_4O_4 \cdot 1/2H_2O$	58.99 (58.79	4.94 5.13	10.39 10.55)	2.04
21-Cu	Gray prisms	275—280	$C_{20}H_{30}CuN_4O_4$	52.85 (52.90	6.58 6.67	12.13 12.34)	—
22-Cu	Gray prisms	230—240	$C_{30}H_{28}CuN_6O_4 \cdot H_2O$	58.40 (58.30	4.87 4.90	13.38 13.60)	1.81





3-Cu: $R^1=H$, $R^2=CH_3$
4-Cu: $R^1=R^2=CH_3$
5-Cu: $R^1=CH_2C_6H_5$, $R^2=CH_3$
6-Cu: $R^1=CHC_2H_5$ (CH_3), $R^2=CH_3$
7-Cu: $R^1=CH_2$-(indol-3-yl), $R^2=CH_3$
14-Cu: $R^1=H$, $R^2=(CH_2)_8CH_3$
15-Cu: $R^1=CH_3$, $R^2=(CH_2)_8CH_3$
16-Cu: $R^1=CH_2C_6H_5$, $R^2=(CH_2)_8CH_3$
17-Cu: $R^1=CH_2H_5$ (CH_3), $R^2=(CH_2)_8CH_3$

18-Cu: R=H
19-Cu: $R=CH_3$
20-Cu: $R=CH_2C_6H_5$
21-Cu: $R=CHC_2H_5$ (CH_3)
22-Cu: $R=CH_2$-(indol-3-yl)

Chart 4

at 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 suggest that these complexes have the structure shown in Chart 4.[14]

The results of antimicrobial activity tests on the 3-acyltetramic acids and their copper complexes are summarized in Table II. None of the 3-acetyltetramic acid derivatives (**3**—**7**) inhibited the growth of *B. subtilis* (IFO-3513) or *S. aureus* (IFO-3061), but all of the 3-decanoyltetramic acid derivatives (**14**—**17**) and their copper complexes (**14**-Cu-**17**-Cu) showed high activity against *B. subtilis* and *S. aureus*. 3-Decanoyltetramic acid (**14**) exhibited the highest activity, and the introduction of a bulky substituent such as a benzyl or 1-methylpropyl group resulted in a decrease in the antimicrobial activity. Conversion of **14** and **15** to their copper complexes, **14**-Cu and **15**-Cu, did not have any marked effect, but conversion of **16** and **17** to **16**-Cu and **17**-Cu resulted in a remarkable increase in activity.

Table II. Antimicrobial Activities of 3-Acyltetramic Acids and Their Copper (II) Complexes (MIC: mcg/ml)

Microorganism	Compound				
	3—**7** 3-Cu—**7**-Cu	**14**	**15**	**16**	**17**
B. subtilis (IFO-3513)	>100	0.75	1.56	25	25
St. aureus (IFO-3061)	>100	6.25	6.25	100	50

Microorganism	Compound			
	14-Cu	**15**-Cu	**16**-Cu	**17**-Cu
B. subtilis (IFO-3513)	12.5	1.56	1.56	6.25
St. aureus (IFO-3061)	25	6.25	6.25	12.5

The 3-acetyl and 3-decanoyltetramic acids did not inhibit the growth of *Escherichia coli*, *Pseudomonas aureginosa*, *Serratia marcescens* or various molds (*Asgergillus niger*, *Penicillium citrinum*, *Cladosporium herbarum*, *Mucor spinescens*) at 100 mcg/ml.

No inhibitory effects of 3-(1-iminoethyl)tetramic acid derivatives (**18**—**22**) and their copper complexes (**18**-Cu—**22**-Cu) on chlorophyll development of rice and radish were observed, although 3-(1-iminoethyl)-5-methyltetronic acid caused distinct chlorosis, as reported in the literature.[3,9]

It may be concluded, therefore, that antimicrobial activity requires a 3-decanoyl group in the molecule, as in the case of the tetronic acid derivatives, and that the substituent at the 5-position also affects the activity. In addition, some of the copper (II) complexes, showed higher activity than their parent compounds. On the other hand, inhibitory activity towards chlorophyll development requires a tetronic acid nucleus in the molecule.

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 spectro-

14) M. Kato, H.B. Jonassen, and J.C. Fanning, *Chem. Rev.*, **64**, 99 (1964).

meter 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.

3-Acetyltetramic Acids——General Method: Diketene was added dropwise to an amino acid ester with stirring and cooling (5°). When the addition was over, the reaction mixture was warmed to room temperature and stirred for 0.5 hr. Excess diketene was evaporated off under reduced pressure to leave the N-acetoacetylamino acid ester, which was added to a methanolic solution of CH_3ONa (prepared from Na metal and methanol) at 20° with stirring. After addition of benzene, the reaction mixture was refluxed for 4 hr and then allowed to stand at room temperature overnight. Water was added to the reaction mixture and the organic layer was separated and extracted three times with water. The original water layer and the extracts were combined and acidified with conc. H_2SO_4 to give pH 2 under cooling. The acidic solution was extracted three times with ether and the extracts were washed with saturated brine. After drying over Na_2SO_4, the solvent was evaporated off *in vacuo* to give crude 3-acetyltetramic acid, which was recrystallized from an appropriate solvent.

3-Acetyltetramic Acid (3)——**3** (5.8 g) was obtained from ethyl glycinate (**2**: $R^1=H$, $R^2=C_2H_5$) (10 g, 97 mmol), diketene (10.1 g; 120 mmol) and Na (2.53 g; 110 mg atom) by the general method as pale orange prisms. mp 154—159° (*lit*[6,11] 155°). IR ν_{max} cm^{-1}: 3250 (OH, NH), 1715 (CO), 1665, 1615 (CONH). NMR ($CDCl_3$) δ: 2.45 (3H, s, $COCH_3$), 3.82, 3.95 (2H, s, $-\overset{|}{C}-C\underline{H}_2-NH-$). *Anal.* Calcd for $C_6H_7NO_3$: C, 51.06; H, 5.01; N, 9.93. Found: C, 51.21; H, 4.93; N, 9.65.

3-Acetyl-5-methyltetramic Acid (4)——**4** (4.03 g) was obtained from ethyl alaninate (**2**: $R^1=CH_3$, $R^2=C_2H_5$) (4.56 g; 39 mmol), diketene (3.95 g; 47 mmol) and Na (0.99 g; 43 mg atom) by the general method as colorless needles. mp 118—119° (*lit*[11] 115—116°). IR ν_{max} cm^{-1}: 3310 (OH, NH), 1700 (CO), 1670, 1620 (CONH). NMR ($CDCl_3$) δ: 1.38 (3H, d, $J=8$ Hz, $-\overset{|}{C}H-C\underline{H}_3$), 2.45 (3H, s, $COCH_3$), 3.90 (1H, q, $J=8$ Hz, $-\overset{|}{C}\underline{H}-CH_3$), 7.05 (1H, s, NH), 12.38 (1H, s, OH).

3-Acetyl-5-benzyltetramic Acid (5)——**5** (4.3 g) was obtained from ethyl phenylalaninate (**2**: $R^1=CH_2C_6H_5$, $R^2=C_2H_5$) (7.53 g; 39 mmol), diketene (3.95 g; 47 mmol) and Na (0.99 g; 43 mg atom) by the general method as colorless needles. mp 155—157° (*lit*[9] 148—153°). IR ν_{max} cm^{-1}: 3180 (OH, NH), 1710 (CO), 1650 (CONH). NMR ($CDCl_3$) δ: 2.45 (3H, s, $COCH_3$), 2.61 (1H, dd, $J=12$, 14 Hz, $C_6H_5-C\underline{H}_2-\overset{|}{C}H-$), 3.33 (1H, dd, $J=4$, 14 Hz, $C_6H_5-C\underline{H}_2-\overset{|}{C}H-$), 4.04 (1H, dd, $J=4$, 12 Hz, $C_6H_5CH_2-\overset{|}{C}\underline{H}-NH$), 6.40 (1H, s, CONH), 7.24 (5H, s, arom.H), 9.20 (1H, s, OH). *Anal.* Calcd for $C_{13}H_{13}NO_3$: C, 67.51; H, 5.68; N, 6.06. Found: C, 67.75; H, 5.62; N, 6.26.

3-Acetyl-5-(1-methylpropyl)tetramic Acid (6)——**6** (9.2 g) was obtained from ethyl isoelucinate (**2**: $R^1=CH(CH_3)C_2H_5$, $R^2=C_2H_5$) (5.2 g; 22 mmol), diketene (5 g, 60 mmol) and Na (1.27 g; 55.2 mg atom) by the general method as a vitcous oil. IR ν_{max}^{neat} cm^{-1}: 3230 (OH, NH), 1705 (CO), 1665, 1620 (CONH). NMR ($CDCl_3$) δ: 0.87 (3H, d, $J=6$ Hz, $-\overset{|}{C}H-C\underline{H}_3$), 1.04 (3H, t, $J=6$ Hz, $-CH_2-C\underline{H}_3$), 1.32 (2H, m, $-\overset{|}{C}H-C\underline{H}_2-CH_3$), 2.0 (1H, m, $-CH_2-\overset{|}{C}\underline{H}-CH_3$), 3.85 (1H, m, $-\overset{|}{C}H-\overset{|}{C}\underline{H}-NH$), 7.30 (1H, m, NH), 12.18 (1H, bs, OH).

3-Acetyl-5-(3-indolylmethyl)tetramic Acid (7)——**7** (3.5 g) was obtained from ethyl tryptophanate (**2**: $R^1=CH_2C_8H_6N$, $R^2=C_2H_5$) (5.2 g; 22 mmol), diketene (2.27 g; 27 mmol) and Na (0.55 g; 24 mg atom) by the general method as colorless prisms. mp 179—180°. IR ν_{max} cm^{-1}: 3280 (OH, NH), 1703 (CO), 1653, 1610 (CONH). NMR (DMSO-d_6) δ: 2.26 (3H, s, $COCH_3$), 3.06 (2H, d, $J=6$ Hz, $=\overset{|}{C}-C\underline{H}_2-\overset{|}{C}H-$), 4.17 (1H, *t*, $J=6$ Hz, $-CH_2-\overset{|}{C}\underline{H}-$), 6.9—7.7 (5H, m, arom.H), 7.95 (1H, s, NH), 8.80 (1H, bs, NH), 10.80 (1H, bs, OH). *Anal.* Calcd for $C_{15}H_{14}N_2O_3$: C, 66.65; H, 5.23; N, 10.37. Found: C, 66.35; H, 5.20; N, 10.44.

Ethyl N-Bromoacetylphenylalaninate (8)——Bromoacetyl bromide (3.63 g; 18 mmol) was added dropwise to a solution of ethyl phenylalaninate (6.8 g; 35 mmol) in dry ether (20 ml) with ice-cooling. When the addition was complete, the mixture was warmed to room temperature and stirred for 2 hr. Precipitates formed were filtered off and washed thoroughly with ether and ethyl acetate. The filtrate and washings were combined, washed with water and saturated brine, then dried over Na_2SO_4. Removal of the solvent gave **8** (5.23 g) as colorless needles, which were recrystallized from isopropanol. mp 76.5—78.0°. IR ν_{max} cm^{-1}: 3300 (NH), 1735 (COO), 1655, 1540 (CONH), 1230, 1200. NMR ($CDCl_3$) δ: 1.25 (3H, t, $J=7$ Hz, $-CH_2-C\underline{H}_3$), 3.15 (2H, d, $J=6$ Hz, $C_6H_5-C\underline{H}_2\overset{|}{C}H-$), 3.83 (2H, s, $COCH_2Br$), 4.18 (2H, q, $J=7$ Hz, $COOC\underline{H}_2CH_3$), 4.82 (1H, d of t, $J=6$, 8 Hz, $CH_2C\underline{H}NH$), 6.9 (1H, m, NH), 7.23 (5H, m, arom.H).

Ethyl N-(3-Oxododecanoyl)phenylalaninate (10)——Granulated Zn (1.6 g; 24.5 mg atom) and copper(II) acetate monohydrate (0.35 g; 1.8 mmol) were added to acetic acid (3 ml), and the resulting suspension was stirred at room temperature under an N_2 atmosphere for 30 min. Acetic acid was then decanted off and the couples were washed with dry ether (5 ml×3) and dry benzene (5 ml). After adding dry THF (5 ml) to the Zn-Cu couples, a solution of ethyl N-bromoacetylphenylalaninate (2 g; 6.4 mmol) and decanal (0.8 g; 5.1 mmol) in dry THF (5 ml) was added dropwise with stirring in such a manner as to keep the mixture at gentle reflux. This took 25 min. After the addition, the mixture was refluxed for 1 hr, then allowed to cool, acidified with 5 N H_2SO_4, and extracted three times with ether. The extracts were washed with saturated brine and dried over Na_2SO_4. Removal of the solvent *in vacuo* gave 2.45 g of crude ethyl N-(3-hydroxydodecanoyl)phenylalaninate (**9**) as a viscous oil. The crude **9** was dissolved in acetone (85 ml) and oxidized with Jones' reagent. The oxidized product obtained by the usual work-up was purified by SiO_2 column

chromatography (acetone–hexane=1: 4—2: 3). From the fractions eluted earlier, **10** (354 mg) was obtained as a gum. IR ν_{max}^{neat} cm^{-1}: 3300 (NH), 3030 (arom), 1740 (COO), 1660 (CO). From the fractions eluted later, ethyl N-acetylphenylalaninate (**11**) (845 mg) was obtained as an oil. IR ν_{max}^{neat} cm^{-1}: 3310 (NH), 3050 (arom), 1745 (COO), 1660 (CONH), 1545, 1210, 1190, 1025. NMR ($CDCl_3$) δ: 1.20 (3H, t, J=7 Hz, $COOCH_2C\underline{H}_3$), 1.93 (3H, s, $COCH_3$), 3.08 (2H, d, J=6 Hz, $C_6H_5C\underline{H}_2\dot{C}H$), 4.13 (2H, q, J=7 Hz, $COOC\underline{H}_2CH_3$), 4.82 (1H, m, $CH_2C\underline{H}NH$), 6.55 (1H, m, CONH), 7.19 (5H, s, arom. H).

5-Benzyl-3-decanoyltetramic Acid (16)——Ethyl N-(3-oxododecanoyl)phenylalaninate (**10**) (354 mg; 0.91 mmol) was treated with a methanolic solution of CH_3ONa (prepared from Na (30 mg; 1.3 mg atom) and methanol (4 ml)). After addition of benzene (3 ml), the reaction mixture was refluxed for 3 hr. Water was added with cooling, and the organic layer was separated and washed twice with water. The original water layer and the washings were combined and acidified with conc.HCl to give pH 2, then extracted three times with ether. The extracts were washed with saturated brine and dried over Na_2SO_4. Removal of the solvent gave a solid which was purified by SiO_2 column chromatography (chloroform–methanol=95: 5) to furnish **16** (170 mg) as colorless needles (recrystallized from benzene–petroleum ether). mp 113—115° IR ν_{max} cm^{-1}: 3040 (arom), 1710 (CO), 1655, 1630 (CONH). NMR ($CDCl_3$) δ: 0.89 (3H, t, J=5 Hz, $CH_2C\underline{H}_3$), 1.29 (14H, s, $COCH_2(C\underline{H}_2)_7CH_3$), 2.65 (1H, dd, J=9, 14 Hz, $C_6H_5C\underline{H}_2\dot{C}H$), 2.85 (2H, t, J=6 Hz, $COCH_2CH_2$), 3.28 (1H, dd, J=4, 14 Hz, $C_6H_5C\underline{H}_2\dot{C}H$), 4.01 (1H, dd, J=4, 9 Hz, $C_6H_5CH_2\dot{C}\underline{H}$), 6.64 (1H, s, CONH), 7.23 (5H, s, arom.H), 11.80 (1H, m, OH). *Anal.* Calcd for $C_{21}H_{29}NO_3$: C, 73.42; H, 8.53; N, 4.08. Found: C, 73.42; H, 8.38; N, 4.20.

3-Oxododecanoyl Chloride (12)——A mixture of anhydrous ethanol (5 ml) and carbon tetrachloride (10 ml) was added to magnesium shavings (17 g; 699 mg atom) in a flask to start the reaction. After addition of ethanol (90 ml), THF (654 ml) was added dropwise at the beginning and then rapidly to maintain a gentle reflux. When the exothermic reaction subsided, the reaction mixture was cooled to room temperature and monoethyl malonate (65.4 mg; 572 mmol) was added dropwise. After refluxing for 6 hr, the mixture was allowed to stand overnight at room temperature. Decanoyl chloride (50 g; 263 mmol) was added dropwise to maintain a gentle reflux, and the mixture was refluxed for 4 hr. HCl (2 N) was added dropwise with cooling until gas evolution ceased, then the organic layer was separated. After extracting the water layer three times with ether, the extracts were combined with the original organic layer, washed with saturated brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was distilled to give ethyl 3-oxododecanoate (53.42 g). bp 125—130° (3 Torr). IR ν_{max}^{neat} cm^{-1}: 1750 (COO), 1725 (CO), 1640, 1225. NMR ($CDCl_3$) δ: 0.90 (3H, t, J=5 Hz, $CH_2C\underline{H}_3$), 1.28 (14H, s, $CH_2(C\underline{H}_2)_7CH_3$), 1.29 (3H, t, J=7 Hz, $COOCH_2C\underline{H}_3$), 2.54 (2H, t, J=6 Hz, $COC\underline{H}_2CH_2$), 3.45 (2H, s, $COCH_2COO$), 4.19 (2H, q, J=7 Hz, $OC\underline{H}_2CH_3$). NaOH solution (1 N, 84.5 ml; 84.5 mmol) was added to a suspension of ethyl 3-oxododecanoate (18.565 g; 76.7 mmol) in water (131 ml) with cooling, and then the mixture was stirred at room temperature for 4.5 hr. After washing with ether to remove the unreacted ester, the water layer was acidified to give pH 3 with H_2SO_4 (1 N), then extracted three times with ether. The extracts were washed with saturated brine, dried over Na_2SO_4 and concentrated *in vacuo* to leave 3-oxododecanoic acid (13.78 g) as colorless plates. IR ν_{max} cm^{-1}: 3200—2400 (COOH), 1710 (CO, COOH), 1240, 1160, 1080, 905. Thionyl chloride (5.738 g; 48 mmol) was added to a suspension of 3-oxododecanoic acid (8.629 g; 40 mmol) in benzene (16 ml) under cooling. After stirring the mixture at room temperature for 5 hr, the solvent and the excess thionyl chloride were evaporated off *in vacuo* at room temperature to give 3-oxododecanoyl chloride (**12**) as an oil; this was used for acylation without purification. IR ν_{max}^{neat} cm^{-1}: 1775 (COCl), 1720 (CO), 1680, 1630, 1590.

Ethyl N-(3-Oxododecanoyl)glycinate (13: R^1=H, R^2=C_2H_5)——A solution of 3-oxododecanoyl chloride (**12**) [prepared from 3-oxododecanoic acid (13 g; 56.7 mmol) and thionyl chloride (8.1 g; 68 mmol) as mentioned above] in ether (30 ml) was added to a solution of ethyl glycinate (**2**: R^1=H, R^2=C_2H_5) (11.67 g; 113 mmol) in ether (40 ml) with stirring and cooling. The mixture was stirred at room temperature for 3 hr, then precipitates formed were filtered off and washed thoroughly with ether. The filtrate and washings were combined and concentrated *in vacuo* to give a viscous oil (19.903 g), which was purified by SiO_2 colum chromatography (ethyl acetate–benzene=4: 5) to furnish **13** (R^1=H, R^2=C_2H_5) (9.365 g) as a crystalline mass. IR ν_{max} cm^{-1}: 3300, 3100 (NH), 1745 (COO), 1715 (CO), 1650 (CONH), 1210. NMR ($CDCl_3$) δ: 0.90 (3H, t, J=6 Hz, $CH_2C\underline{H}_3$), 1.28 (14 Hz, s, $CH_2(C\underline{H}_2)_7CH_3$), 1.29 (3H, t, J=7 Hz, $OCH_2C\underline{H}_3$), 2.55 (2H, t, J=6 Hz, $COC\underline{H}_2CH_2$), 3.46 (2H, s, $COCH_2CO$), 3.99, 4.08 (2H, s, $COC\underline{H}_2NH$), 4.21 (2H, q, J=7 Hz, $COOC\underline{H}_2$-CH_3), 7.48 (1H, bs, CONH).

3-Decanoyltetramic Acid (14)——A solution of ethyl N-(3-oxododecanoyl)glycinate (**13**: R^1=H, R^2=C_2H_5) in dry benzene (62.5 ml) was added to a methanolic solution of CH_3ONa [prepared from Na (1.13 g; 49.3 mg atom) and methanol (100 ml)] with stirring at room temperature. After refluxing the reaction mixture for 3 hr, water was added to the cooled solution. The organic layer was separated and extracted three times with water. The original water layer and the extracts were combined, acidified with conc. HCl to give pH 2 and then extracted three times with ether. The extracts were washed with saturated brine, dried over Na_2SO_4 and concentrated *in vacuo* to leave **14** (6.94 g) as a crystalline powder, which was recrystallized from benzene–petroleum ether. mp 107—110°. IR ν_{max} cm^{-1}: 3150, 3080 (OH, NH), 1708 (CO), 1645, 1600 (CONH). NMR ($CDCl_3$) δ: 0.89 (3H, t, J=5 Hz, $CH_2C\underline{H}_3$), 1.29 (14H, s, $CH_2(C\underline{H}_2)_7CH_3$),

2.86 (2H, t, J=7 Hz, $COC\underline{H}_2CH_2$), 3.82, 3.95 (2H, s, $CC\underline{H}_2NH$), 6.85 (1H, s, $CH_2N\underline{H}CO$), 12.25 (1H, m, OH). *Anal.* Calcd for $C_{14}H_{28}NO_3$: C, 66.36; H, 9.17; N, 5.53. Found: C, 66.14; H, 8.92; N, 5.53.

3-Decanoyl-5-methyltetramic Acid (15)——Methyl N-(3-oxododecanoyl)alaninate (**13**: R^1=R^2=CH_3) (3.397 g) was obtained as a viscous oil from **2** (R^1=R^2=CH_3) (5.23 g; 50.8 mmol) and **12** (5.9 g; 25.4 mmol), using the procedure described for **13** (R^1=H, R^2=C_2H_5), followed by SiO_2 column chromatography (benzene–ethyl acetate=1: 1). IR ν_{max}^{neat} cm^{-1}: 3300 (NH), 1750 (COO), 1722 (CO), 1650 (CONH). **15** (1.916 g) was obtained as a viscous oil from the β-keto amide **13** (R^1=R^2=CH_3) 2.268 g; 7.6 mmol) and Na (267 mg; 11.4 mg atom) using the same procedure as for **14**. After storage in a refrigerator, it was recrystallized from benzene–petroleum ether. mp 57—58°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3470, 3240 (OH, NH), 1715 (CO), 1665, 1618 (CONH). NMR ($CDCl_3$) δ: 0.88 (3H, t, J=5 Hz, $CH_2C\underline{H}_3$), 1.28 (14H, s, $CH_2(C\underline{H}_2)_7CH_3$), 1.38 (3H, d, J=6 Hz, $CHC\underline{H}_3$), 2.84 (2H, t, J=7 Hz, $COC\underline{H}_2CH_2$), 3.88 (1H, q, J=6 Hz, $-C\underline{H}-CH_3$), 6.90 (1H, m, $CHN\underline{H}$), 12.42 (1H, s, OH). *Anal.* Calcd for $C_{15}H_{25}NO_3$: C, 67.37; H, 9.44; N, 5.24. Found: C, 67.41; H, 9.42; N, 5.53.

5-Benzyl-3-decanoyltetramic Acid (16)——Methyl N-(3-oxododecanoyl)phenylalaninate (**13**: R^1=$CH_2C_6H_5$, R^2=CH_3) (11.189 g) was obtained from **2** (R^1=$CH_2C_6H_5$, R^2=CH_3) (16.85 g; 102 mmol) and **12** (11.857 g; 51 mmol) as a viscous oil using the same procedure as for **13** (R^1=H, R^2=C_2H_5), followed by SiO_2 column chromatography ($CHCl_3$). IR ν_{max}^{neat} cm^{-1}: 3300 (NH), 3030 (arom), 1750 (COO), 1655 (CONH). NMR ($CDCl_3$) δ: 0.89 (3H, t, J=5 Hz, $CH_2C\underline{H}_3$), 1.26 (14H, s, $CH_2(C\underline{H}_2)_7CH_3$), 2.45 (2H, t, J=7 Hz, $COC\underline{H}_2CH_2$), 3.10 (2H, d, J=6 Hz, $C_6H_5C\underline{H}_2CH$), 3.33 (2H, s, $COCH_2CO$), 3.68 (3H, s, $COOCH_3$), 4.84 (1H, q, J=6 Hz, $CH_2C\underline{H}NH$), 7.17 (5H, s, arom.H). **16** (3.82 g) was synthesized from the β-keto amide **13** (R^1=$CH_2C_6H_5$, R^2=CH_3) and Na (938 mg; 40.8 mg atom) using the same procedure as for **14**. The resulting colorless needles were recrystallized from benzene–petroleum ether. mp 114—115°. The product was identical in terms of IR and NMR spectra with that obtained by the Reformatsky reaction.

3-Decanoyl-5-(1-methylpropyl)tetramic Acid (17)——Methyl N-(3-oxododecanoyl)isoleucinate (**13**: R^1=$CH(CH_3)C_2H_5$, R^2=CH_3) (3.98 g) was obtained from **2** (R^1=$CH(CH_3)C_2H_5$, R^2=CH_3) (4.158 g; 28.7 mmol) and **12** (3.334 g; 14.3 mmol) as a viscous oil using the same procedure as for **13** (R^1=H, R^2=C_2H_5), followed by SiO_2 column chromatography (ethyl acetate–benzene=4: 5). IR ν_{max}^{neat} cm^{-1}: 3330 (NH), 1745 (COO), 1720 (CO), 1650, 1535 (CONH). **17** (3.22 g) was synthesized from the β-keto amide **13** (R^1=$CH(CH_3)C_2H_5$, R^2=CH_3) (3.98 g; 11.7 mmol) and Na (403 mg; 17.5mg atom) using the same procedure as for **14**. The product was a viscous oil. IR ν_{max} cm^{-1}: 3180, 3070 (OH, NH), 1700 (CO), 1658, 1618 (CONH). NMR ($CDCl_3$) δ: 0.86 (3H, t, J=6 Hz, $CH_2C\underline{H}_3$), 0.93 (3H, d, J=5 Hz, $-C\underline{H}CH_3$), 0.98 (3H, t, J=6 Hz, $CHCH_2C\underline{H}_3$), 1.28—1.7 (17H, bs, $CH_2C\underline{H}CH_3$, $CH_3C\underline{H}_2CHCH_3$, $COCH_2(C\underline{H}_2)_7CH_3$), 2.85 (2H, bt, J=7 Hz, $COC\underline{H}_2CH_2$), 3.38 (1H, dd, J=3, 5 Hz, $=CC\underline{H}CHCH_3$), 7.10 (1H, m, CONH), 11.45 (1H, m, OH).

Imination of 3-Acetyltetramic Acids——General Method: 3-Acetyltetramic acids 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 an appropriate solvent.

3-(1-Iminoethyl)tetramic Acid (18)——**18** (1.2 g) was obtained from **2** (2 g; 14 mmol) by the general method as colorless prisms (recrystallized from methanol–ethyl acetate). mp 238—240°. IR ν_{max} cm^{-1}: 3340, 3300 (OH, NH), 1680 (CO), 1640, 1530 (CONH). NMR (DMSO-d_6) δ: 2.35 (3H, s, $N=CCH_3$), 3.50, 3.57 (2H, s, $=CC\underline{H}_2NH$), 7.10, 7.34, 8.75, 9.50 (3H, m, C=NH, CONH, OH). *Anal.* Calcd for $C_6H_8N_2O_2$: C, 51.42; H, 5.75; N, 19.99. Found: C, 51.21; H, 5.74; N, 19.79.

3-(1-Iminoethyl)-5-methyltetramic Acid (19)——**19** (2.87 g) was obtained from **3** (3 g, 19.4 mmol) by the general method as colorless prisms (recrystallized from methanol). mp 218.5—221°. IR ν_{max} cm^{-1}: 3310, 3250, 3105 (OH, NH), 1680 (CO), 1635, 1530 (CONH). NMR (DMSO-d_6) δ: 1.15 (3H, d, J=8 Hz, $CHC\underline{H}_3$), 2.35 (3H, s, $N=CCH_3$), 3.52 (1H, q, J=8 Hz, $C\underline{H}CH_3$), 7.35, 8.7, 9.5 (each 1H, bs, C=NH, CNHCO, OH). *Anal.* Calcd for $C_7H_{10}N_2O_2$: C, 54.53; H, 6.54; N, 18.14. Found: C, 54.30; H, 6.51; N, 17.96.

5-Benzyl-3-(1-iminoethyl)tetramic Acid (20)——**20** (1.4 g) was obtained from **4** (2 g; 8.7 mmol) by the general method as colorless prisms (recrystallized from methanol). mp 157—159°. IR ν_{max} cm^{-1}: 3300, 3150 (OH, NH), 1685 (CO), 1620, 1520 (CONH). NMR (DMSO-d_6) δ: 2.29 (3H, s, $N=CCH_3$), 2.86 (2H, m, $C_6H_5C\underline{H}_2CH$), 3.88 (1H, m, $C_6H_5CH_2C\underline{H}$), 7.19 (5H, s, arom.H), 8.60, 9.35, 9.63 (each 1H, bs, C=NH, CNHCO, OH). *Anal.* Calcd for $C_{13}H_{14}N_2O_2$: C, 67.80; H, 6.14; N, 12.17. Found: C, 67.89; H, 6.08; N, 12.40.

3-(1-Iminoethyl)-5-(1-methylpropyl)tetramic Acid (21)——**21** (1.6 g) was obtained from **5** (5.6 g, 28 mmol) by the general method as colorless needles (recrystallized from ethyl acetate). mp 153—156°. IR ν_{max} cm^{-1}: 3280, 3130 (OH, NH), 1673 (CO), 1620, 1530 (CONH). NMR (DMSO-d_6) δ: 0.72 (3H, t, J=6 Hz, $CH_2C\underline{H}_3$), 0.92 (3H, d, J=6 Hz, $CHC\underline{H}_3$), 0.8—1.9 (3H, m, $C\underline{H}C\underline{H}_2CH_3$), 2.35 (3H, s, $N=CCH_3$), 3.54 (1H, m, $-C\underline{H}CHNH$), 7.29, 7.50, 8.68, 9.45, 9.70 (3H, m, CONH, C=NH, OH). *Anal.* Calcd for $C_{10}H_{16}N_2O_2$: C, 59.82; H, 8.05; N, 13.96. Found: C, 59.57; H, 8.13; N, 13.91.

3-(1-Iminoethyl)-5-(3-indolylmethyl)tetramic Acid (22)——**22** (1.4 g) was obtained from **6** (2 g; 7.4 mmol) by the general method as colorless prisms (recrystallized from ethyl acetate–hexane). mp 140—146°. IR ν_{max} cm^{-1}: 3290, 3170 (OH, NH), 1710 (CO), 1630, 1520 (CONH). NMR (DMSO-d_6) δ: 2.28 (3H, s, $N=CCH_3$), 3.0 (2H, m, $=CC\underline{H}_2CH$), 3.90 (1H, m, $=CCH_2C\underline{H}NH$), 6.8—7.7 (5H, m, arom.H), 8.65, 9.35, 9.63, 10.75 (4H, bs, CONH; C=NH, OH, NH). *Anal.* Calcd for $C_{15}H_{15}N_3O_2 \cdot 1/2H_2O$: C, 64.72; H 5.81. Found: C, 64.92; H, 5.77.

Copper(II) Complexes of 3-Acyl and 3-(1-Iminoethyl)tetramic Acids——General Method: A tetramic 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 an appropriate solvent. Physical data for the products are listed in Table I.

Antimicrobial Test——Antimicrobial activity of a test compound 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, and solidified. A loopful of the test organism suspension was streaked on the agar plate and the plate was incubated at 33° for 18—20 hr. The antimicrobial activity was expressed as the minimum inhibitory concentration (MIC, mcg/ml).

Inhibition Test for Chlorophyll Development——The test compound was suspended in an aqueous solution containing Tween 20 and acetone to give various concentrations (10, 100 mcg/ml). The solution (5 ml) was poured onto a filter paper (Toyo No. 2) in a Petri dish (diameter, 9 cm). Twenty radish seeds were put on the filter paper and cultured under 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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