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Syntheses and Antimicrobial Activities of 3-Acyltetramic Acid Derivatives

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3-Acyltetramic acids having various substituents at the 1- and 5-positions, and possessing a tricarbonylmethane structure, were synthesized and tested for antimicrobial activity to investigate the structure-activity relationships. It was found that the nature of the substituents at the 1- and 5-positions as well as the 3-position is an important factor for activity to inhibit the growth of *Bacillus subtilis* and *Staphylococcus aureus*.

Keywords—structure-activity relationship; 3-acyltetramic acid derivative; antimicrobial activity; tricarbonylmethane structure; ethyl 3-oxoalkanoate

Tenuazoic acid,¹⁾ streptolydigin,²⁾ tirandamycin³⁾ and malonomycin,⁴⁾ which are typical antibiotics, all possess a 3-acyltetramic acid moiety as a tricarbonylmethane structure. In the previous paper,⁵⁾ we reported the synthesis of 5-substituted 3-acetyl- and 3-decanoyltetramic acids and their copper (II) complexes, which also possess the tricarbonylmethane structure. Testing 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 3515 and *Staphylococcus aureus* IFO 3061; further, the introduction of a bulky substituent such as a benzyl or 1-methylpropyl group at the 5-position resulted in a decrease in the antimicrobial activity.

We therefore synthesized 3-acyltetramic acids having various substituents at the 1- and 5-positions and tested the antimicrobial activity of the compounds to investigate the structure-activity relationships in more detail.

3-Acetyltetramic acid derivatives (**2**—**6**) were synthesized in mediocre yield by treatment of amino acid esters (**1a**—**e**) with diketene followed by cyclization of the resulting amides by base, according to the well-known Lacy method.^{6,7)} Other 3-acyltetramic acid derivatives (**9**—**32**) were prepared similarly by cyclization of the crude β -keto amides (**8**), which were derived from the amino acid esters (**1a**—**e**) and appropriately 4-substituted ethyl acetoacetates (**7a**—**i**) by heating in xylene, in the presence of sodium methoxide in xylene and methanol in moderate yields.

The structures of the newly obtained compounds were confirmed by elemental analyses or high-resolution mass spectroscopy, and infrared (IR) and nuclear magnetic resonance (NMR) spectral analyses. All the 3-acyltetramic acid derivatives exist mostly as the enol form shown in Chart 1, because their IR spectra show a broad band due to the OH group at 3400—2600 cm⁻¹ and the NMR spectra have a broad signal at 11—14 ppm. The carbonyl absorption band due to the 3-acyl group appears near 1620 cm⁻¹.

The results of the antimicrobial activity tests on the 3-acyltetramic acid derivatives are summarized in Table I. Some of these compounds inhibited the growth of *B. subtilis* and *St. aureus*, but did not show any activities against *Escherichia coli*, *Pseudomonas aeruginosa*,

TABLE I. Physical Properties and Antimicrobial Activities of 3-Acyltetramic Acids

Compd.	R ¹	R ²	R ³	Yield (%)	mp (°C) or bp (°C) ^{a)} (Torr)	Formula	Analysis (%) or MS (M ⁺ : m/z) Found (Calcd)			Antimicrobial activities (MIC: µg/ml)	
							C	H	N	<i>B. subtilis</i>	<i>St. aureus</i>
2 ^{b)}	CH ₃		H	Quant.	51—52	C ₇ H ₉ NO ₃	155.0561 (155.0582)			100	100
9	CH ₃	(CH ₂) ₆ CH ₃	H	49	189—191 (0.6)	C ₁₃ H ₂₁ NO ₃	239.1508 (239.1520)			50	25
10	CH ₃	(CH ₂) ₈ CH ₃	H	38	164—166 (0.6)	C ₁₅ H ₂₅ NO ₃	267.1835 (267.1835)			1.56	6.25
11	CH ₃	(CH ₂) ₁₀ CH ₃	H	16	36—37	C ₁₇ H ₂₉ NO ₃	69.11 (69.11)	9.93 9.90	4.73 4.74	1.56	1.56
12	CH ₃	(CH ₂) ₁₂ CH ₃	H	16	187—188 (0.6)	C ₁₉ H ₃₃ NO ₃	323.2436 (323.2459)			100	100
13	CH ₃	CH=CH(CH ₂) ₆ CH ₃	H	15	170—172 (0.6)	C ₁₅ H ₂₃ NO ₃	265.1683 (265.1678)			1.56	12.5
14	CH ₃	(CH ₂) ₃ C ₆ H ₅	H	53	194—196 (0.6)	C ₁₅ H ₁₇ NO ₃	259.1224 (259.1208)			25	50
15	CH ₃	CH(C ₆ H ₅) ₂	H	20	146—147	C ₁₉ H ₁₇ NO ₃	73.97 (74.25)	5.53 5.58	4.62 4.56	6.25	6.25
3	CH ₃		CH ₃	44	91—93 (0.7)	C ₈ H ₁₁ NO ₃	56.05 (56.79)	6.46 6.55	8.14 8.28	100	100
16	CH ₃	(CH ₂) ₄ CH ₃	CH ₃	71	118—120 (0.6)	C ₁₂ H ₁₉ NO ₃	225.1390 (225.1365)			100	50
17	CH ₃	(CH ₂) ₆ CH ₃	CH ₃	40	151—154 (0.7)	C ₁₄ H ₂₃ NO ₃	253.1679 (253.1678)			12.5	12.5
18	CH ₃	(CH ₂) ₈ CH ₃	CH ₃	37	181—183 (0.7)	C ₁₆ H ₂₇ NO ₃	281.1965 (281.1990)			6.25	6.25
19	CH ₃	(CH ₂) ₁₀ CH ₃	CH ₃	13	238—239 (0.8)	C ₁₈ H ₃₁ NO ₃	309.2295 (309.2305)			12.5	12.5

20	CH ₃	(CH ₂) ₁₂ CH ₃	CH ₃	14	189—191 (0.6)	C ₂₀ H ₃₅ NO ₃	337.2597 (337.2615)	100	100
21	CH ₃	(CH ₂) ₃ C ₆ H ₅	CH ₃	37	185—186 (0.6)	C ₁₆ H ₁₉ NO ₃	273.1367 (273.1365)	25	25
22	CH ₃	CH(C ₆ H ₅) ₂	CH ₃	26	113—114	C ₂₀ H ₁₉ NO ₃	74.96 (74.74)	12.5	12.5
4 ⁷⁾	CH ₂ C ₆ H ₅		H	26	74—76	C ₁₃ H ₁₃ NO ₃	5.96 (5.65)	100	100
23	CH ₂ C ₆ H ₅	(CH ₂) ₂ CH ₃	H	66	74	C ₁₅ H ₁₇ NO ₃	6.02 (6.06)	25	25
24	CH ₂ C ₆ H ₅	(CH ₂) ₄ CH ₃	H	20	190—192 (0.7)	C ₁₇ H ₂₁ NO ₃	6.59 (6.61)	6.25	6.25
25	CH ₂ C ₆ H ₅	(CH ₂) ₆ CH ₃	H	71	52.5—53	C ₁₉ H ₂₅ NO ₃	287.1515 (287.1520)	12.5	6.25
26	CH ₂ C ₆ H ₅	(CH ₂) ₈ CH ₃	H	29	214—216 (0.6)	C ₂₁ H ₂₉ NO ₃	72.37 (72.35)	50	12.5
5	CH ₂ C ₆ H ₅		CH ₃	52	144—146 (0.6)	C ₁₄ H ₁₅ NO ₃	343.2139 (343.2146)	100	100
27	CH ₂ C ₆ H ₅	(CH ₂) ₂ CH ₃	CH ₃	56	160—163 (0.6)	C ₁₆ H ₁₉ NO ₃	6.15 (6.16)	50	50
28	CH ₂ C ₆ H ₅	(CH ₂) ₄ CH ₃	CH ₃	35	150—153 (0.5)	C ₁₈ H ₂₃ NO ₃	273.1365 (273.1365)	50	25
29	CH ₂ C ₆ H ₅	(CH ₂) ₆ CH ₃	CH ₃	19	95—98	C ₂₀ H ₂₇ NO ₃	301.1674 (301.1676)	100	6.25
30	CH ₂ C ₆ H ₅	(CH ₂) ₈ CH ₃	CH ₃	23	164—166	C ₂₂ H ₃₁ NO ₃	329.1962 (329.1990)	50	50
6 ⁸⁾	CH ₂ C ₆ H ₅		C ₆ H ₅	50	129—130	C ₁₉ H ₁₇ NO ₃	357.2303 (357.2303)	25	50
31	CH ₂ C ₆ H ₅	(CH ₂) ₂ CH ₃	C ₆ H ₅	49	115—118	C ₂₁ H ₂₁ NO ₃	5.47 (5.58)	12.5	25
32	CH ₂ C ₆ H ₅	(CH ₂) ₄ CH ₃	C ₆ H ₅	28	93—94	C ₂₃ H ₂₅ NO ₃	75.03 (75.20)	50	50
							75.82 (76.00)	3.76 (3.85)	

a) Oven temperature in bulb-to-bulb distillation.

- a : R¹ = CH₃; R³ = H
 b : R¹ = CH₃; R³ = CH₃
 c : R¹ = CH₂C₆H₅; R³ = H
 d : R¹ = CH₂C₆H₅; R³ = CH₃
 e : R¹ = CH₂C₆H₅; R³ = C₆H₅

- a : R² = (CH₂)₂CH₃ f : R² = (CH₂)₁₂CH₃
 b : R² = (CH₂)₄CH₃ g : R² = CH=CH(CH₂)₆CH₃
 c : R² = (CH₂)₆CH₃ h : R² = (CH₂)₃C₆H₅
 d : R² = (CH₂)₈CH₃ i : R² = CH(C₆H₅)₂
 e : R² = (CH₂)₁₀CH₃

Chart 1

Serratia marcescens or various molds (*Aspergillus niger*, *Penicillium citrinum*, *Cladosporium herbarum*, *Mucor spinescens*) at 100 µg/ml.

In the first series (R¹ = CH₃ and R³ = H) (**2** and **9—12**), the highest activity was found with **10** (R² = (CH₂)₈CH₃; molecular weight (MW) 267) and **11** (R² = (CH₂)₁₀CH₃; MW 295); the mean molecular weight of **10** and **11** is 281. In the second series (R¹ = CH₃ and R³ = CH₃) (**3** and **16—20**), the highest activity was found with **18** (R² = (CH₂)₈CH₃; MW 281). Compound **24** (R¹ = CH₂C₆H₅, R² = (CH₂)₄CH₃, and R³ = H; MW 287) showed the highest activity in the third series (R¹ = CH₂C₆H₅, R³ = H) (**4** and **23—26**). Among **5** and **27—30** (R¹ = CH₂C₆H₅ and R³ = CH₃), the highest activity was found with **27** (R² = (CH₂)₂CH₃; MW 273) and **28** (R² = (CH₂)₄CH₃; MW 301) (mean MW of **27** and **28**, 287) against *B. subtilis*, and with compound **29** (R² = (CH₂)₆CH₃; MW 329) against *St. aureus*. In the last series (R¹ = CH₂C₆H₅ and R³ = C₆H₅) (**6**, **31** and **32**), the highest activity was found with **31** (R² = (CH₂)₂CH₃; MW 349) against both microorganisms.

It may be concluded that the antimicrobial activity against *B. subtilis* and *St. aureus* requires a compound with a molecular weight of 280—290 in the case of R³ = H or CH₃ among the tested compounds. In addition, the introduction of a carbon-carbon double bond on the side chain at the 3-position (compare **10** with **13**) did not affect the activity, but substitution with a phenyl group decreased the activity considerably (compare **10** with **14** and **18** with **21**) or only slightly (compare **10** with **15** and **18** with **22**).

TABLE II. IR and NMR Data for 3-Acyltetramic Acid Derivatives

Compd.	IR $\nu_{\max}$ cm^{-1} :	NMR (CDCl_3) δ :
2	(Nujol) 1710, 1650, 1605, 1230, 1200, 1145, 970, 895	2.42 (3H, s, COCH_3), 3.02 (3H, s, NCH_3), 3.73 (2H, s, NCH_2C), 13.19 (1H, s, OH)
3	(Film) 1717, 1640, 1612, 1240, 1160, 920, 860	1.35 (3H, d, $J=7$ Hz, CHCH_3), 2.42 (3H, s, COCH_3), 2.98 (3H, s, NCH_3), 3.72 (1H, q, $J=7$ Hz, CHCH_3), 13.03 (1H, br s, OH)
4	(Nujol) 1715, 1635, 1600, 1240, 1190, 980, 950, 925	2.45 (3H, s, COCH_3), 3.60 (2H, s, NCH_2C), 4.60 (2H, s, NCH_2Ar), 11.65 (1H, br s, OH)
5	(Film) 1715, 1630, 1230, 1165, 1015, 915	1.30 (3H, d, $J=7$ Hz, CHCH_3), 2.46 (3H, s, COCH_3), 3.63 (1H, q, $J=7$ Hz, NCHCH_3), 4.14 and 5.14 (each 1H, d, $J=15$ Hz, NCH_2Ar), 7.2—7.4 (5H, m, Ar-H)
6	(Nujol) 1710, 1620, 1255, 1212, 1185, 1155, 970, 930	2.43 (3H, s, COCH_3), 3.70 and 5.30 (each 1H, d, $J=15$ Hz, NCH_2Ar), 4.51 (1H, s, $\text{NCH}(\text{Ar})\text{C}$), 7.0—7.5 (10H, m, Ar-H), 11.45 (1H, br s, OH)
9	(Film) 1710, 1660, 1620, 1240, 1170, 1100, 1035, 980, 915	0.90 (3H, t, $J=5$ Hz, CH_2CH_3), 1.30 (10H, br s, $\text{COCH}_2-(\text{CH}_2)_5\text{CH}_3$), 2.84 (2H, t, $J=7$ Hz, COCH_2CH_2), 3.03 (3H, s, NCH_3), 3.75 (2H, s, NCH_2C), 11.67 (1H, br s, OH)
10	(Film) 1715, 1655, 1625, 1242, 1165, 1035, 980, 925	0.90 (3H, t, $J=5$ Hz, CH_2CH_3), 1.30 (14H, br s, $\text{COCH}_2-(\text{CH}_2)_7\text{CH}_3$), 2.85 (2H, t, $J=7$ Hz, COCH_2CH_2), 3.03 (3H, s, NCH_3), 3.74 (2H, s, NCH_2C)
11	(Nujol) 1715, 1650, 1610, 1245, 1145, 890	0.89 (3H, t, $J=4$ Hz, CH_2CH_3), 1.28 (18H, br s, $\text{COCH}_2-(\text{CH}_2)_9\text{CH}_3$), 2.83 (2H, t, $J=7$ Hz, COCH_2CH_2), 3.02 (3H, s, NCH_3), 3.72 (2H, s, NCH_2C), 11.55 (1H, br s, OH)
12	(Nujol) 1710, 1655, 1620, 1235, 1160, 925	0.89 (3H, t, $J=5$ Hz, CH_2CH_3), 1.27 (22H, br s, $\text{COCH}_2-(\text{CH}_2)_{11}\text{CH}_3$), 2.83 (2H, t, $J=7$ Hz, COCH_2CH_2), 3.02 (3H, s, NCH_3), 3.73 (2H, s, NCH_2C), 9.05 (1H, br s, OH)
13	(Film) 1710, 1650, 1590, 1245, 980, 900	0.89 (3H, t, $J=5$ Hz, CH_2CH_3), 1.32 (10H, br s, $=\text{CHCH}_2-(\text{CH}_2)_5\text{CH}_3$), 2.30 (2H, m, $=\text{CHCH}_2\text{CH}_2$), 3.02 (3H, s, NCH_3), 3.72 (2H, s, NCH_2C), 7.05—7.30 (2H, m, $\text{COCH}=\text{CHCH}_2$), 12.65 (1H, br s, OH)
14	(Film) 1715, 1655, 1620, 1245, 1165, 985, 910	1.98 (2H, m, $\text{COCH}_2\text{CH}_2\text{Ar}$), 2.75 (4H, m, $\text{COCH}_2\text{CH}_2\text{CH}_2\text{Ar}$), 2.98 (3H, s, NCH_3), 3.68 (2H, s, NCH_2C), 7.21 (5H, s, Ar-H), 13.20 (1H, s, OH)
15	(Nujol) 1732, 1645, 1255, 985, 960	2.95 (3H, s, NCH_3), 3.70 (2H, s, NCH_2C), 6.30 (1H, s, COCHAr_2), 7.30 (10H, s, Ar-H), 12.43 (1H, s, OH)
16	(Film) 1700, 1620 (br), 1230, 1180, 1150, 1060, 920	0.90 (3H, t, $J=5$ Hz, CH_2CH_3), 1.35 (3H, d, $J=7$ Hz, CHCH_3), 1.2—1.6 (6H, m, $\text{COCH}_2(\text{CH}_2)_3\text{CH}_3$), 2.83 (2H, t, $J=7$ Hz, COCH_2CH_2), 2.98 (3H, s, NCH_3), 3.70 (1H, q, $J=7$ Hz, NCHCH_3), 11.70 (1H, br s, OH)
17	(Film) 1710, 1660, 1620, 1242, 1170, 1100, 1035, 980, 915	0.88 (3H, t, $J=5$ Hz, CH_2CH_3), 1.35 (3H, d, $J=6$ Hz, CHCH_3), 1.2—1.5 (10H, m, $\text{COCH}_2(\text{CH}_2)_5\text{CH}_3$), 2.83 (2H, t, $J=7$ Hz, COCH_2CH_2), 2.98 (3H, s, NCH_3), 3.70 (1H, q, $J=6$ Hz, NCHCH_3), 12.03 (1H, br s, OH)
18	(Film) 1715, 1660, 1625, 1235, 1060, 920, 750	0.90 (3H, t, $J=5$ Hz, CH_2CH_3), 1.27 (14H, br s, $\text{COCH}_2-(\text{CH}_2)_7\text{CH}_3$), 1.30 (3H, d, $J=7$ Hz, CHCH_3), 2.83 (2H, t, $J=7$ Hz, COCH_2CH_2), 2.98 (3H, s, NCH_3), 3.70 (1H, q, $J=7$ Hz, NCHCH_3)
19	(Film) 1711, 1650, 1623, 1232, 1162, 1060, 920	0.89 (3H, t, $J=5$ Hz, CH_2CH_3), 1.35 (3H, d, $J=7$ Hz, CHCH_3), 1.27 (18H, br s, $\text{COCH}_2(\text{CH}_2)_9\text{CH}_3$), 2.82 (2H, t, $J=7$ Hz, COCH_2CH_2), 2.97 (3H, s, NCH_3), 3.69 (1H, q, $J=7$ Hz, NCHCH_3), 12.90 (1H, s, OH)
20	(Film) 1710, 1650, 1615, 1230, 1165, 1060, 920	0.89 (3H, t, $J=5$ Hz, CH_2CH_3), 1.25 (22H, s, $\text{COCH}_2-(\text{CH}_2)_{11}\text{CH}_3$), 1.35 (3H, d, $J=7$ Hz, NCHCH_3), 2.81 (2H, t, $J=7$ Hz, COCH_2CH_2), 2.96 (3H, s, NCH_3), 3.68 (1H, q, $J=7$ Hz, NCHCH_3), 10.30 (1H, br s, OH)
21	(Film) 1712, 1652, 1725, 1235,	1.35 (3H, d, $J=7$ Hz, CHCH_3), 1.8—2.2 (2H, m, $\text{COCH}_2\text{CH}_2-$

TABLE II. (continued)

Compd.	IR $\nu_{\max}$ cm^{-1} :	NMR (CDCl_3) δ :
	1162, 1065, 920	CH_2Ar), 2.84 (3H, s, NCH_3), 3.65 (1H, q, $J=7$ Hz, NCH_2CH_3), 7.20 (5H, s, Ar-H), 12.60 (1H, br s, OH)
22	(Nujol) 1725, 1635, 1300, 1265, 1250, 1150, 1065, 975, 925	1.35 (3H, d, $J=7$ Hz, CHCH_3), 2.95 (3H, s, NCH_3), 3.70 (1H, q, $J=7$ Hz, NCH_2CH_3), 6.31 (1H, s, COCHAr_2), 7.32 (10H, m, Ar-H), 12.29 (1H, br s, OH)
23	(Nujol) 1720, 1645, 1600, 1230, 1200, 1105, 905	1.00 (3H, t, $J=6$ Hz, CH_2CH_3), 1.5—1.9 (2H, m, $\text{COCH}_2\text{-CH}_2\text{CH}_3$), 2.83 (2H, t, $J=7$ Hz, COCH_2CH_2), 3.60 (2H, s, NCH_2C), 4.60 (2H, s, NCH_2Ar), 7.30 (5H, s, Ar-H), 10.75 (1H, br s, OH)
24	(Film) 1710, 1650, 1615, 1235, 1065, 1015, 982	0.90 (3H, t, $J=6$ Hz, CH_2CH_3), 1.4 (6H, br s, $\text{COCH}_2\text{-(CH}_2)_3\text{CH}_3$), 2.84 (2H, t, $J=7$ Hz, COCH_2CH_2), 3.59 (2H, s, NCH_2C), 4.58 (2H, s, NCH_2Ar), 7.28 (5H, s, Ar-H)
25	(Film) 1710, 1645, 1600, 1230, 1190, 1120, 1070, 985, 920	0.89 (3H, t, $J=5$ Hz, CH_2CH_3), 1.30 (10H, m, $\text{COCH}_2\text{-(CH}_2)_5\text{CH}_3$), 2.84 (2H, t, $J=7$ Hz, COCH_2CH_2), 3.59 (2H, s, NCH_2C), 4.58 (2H, s, NCH_2Ar), 7.28 (5H, s, Ar-H), 11.58 (1H, br s, OH)
26	(Film) 1705, 1610 (br), 1240, 985, 940	0.89 (3H, t, $J=5$ Hz, CH_2CH_3), 1.28 (14H, br s, $\text{COCH}_2\text{-(CH}_2)_7\text{CH}_3$), 2.82 (2H, t, $J=7$ Hz, COCH_2CH_2), 3.58 (2H, s, NCH_2C), 4.58 (2H, s, NCH_2Ar), 7.26 (5H, s, Ar-H), 12.82 (1H, br s, OH)
27	(Film) 1705, 1616 (br), 1215, 1155, 1055, 905	1.00 (3H, t, $J=6$ Hz, CH_2CH_3), 1.29 (3H, d, $J=7$ Hz, NCHCH_3), 1.70 (2H, m, $\text{COCH}_2\text{CH}_2\text{CH}_3$), 2.83 (2H, t, $J=7$ Hz, COCH_2CH_2), 3.62 (1H, q, $J=7$ Hz, NCH_2CH_3), 4.12 and 5.11 (each 1H, d, $J=15$ Hz, NCH_2Ar), 7.30 (5H, s, Ar-H), 11.30 (1H, br s, OH)
28	(Film) 1700, 1615 (br), 1230, 1075, 930	0.91 (3H, t, $J=5$ Hz, CH_2CH_3), 1.30 (3H, d, $J=7$ Hz, NCHCH_3), 1.2—1.7 (6H, m, $\text{COCH}_2\text{(CH}_2)_3\text{CH}_3$), 2.85 (2H, t, $J=7$ Hz, COCH_2CH_2), 3.62 (1H, q, $J=7$ Hz, NCH_2CH_3), 4.12 and 5.11 (each 1H, d, $J=15$ Hz, NCH_2Ar), 7.30 (5H, s, Ar-H), 11.00 (1H, br s, OH)
29	(Film) 1705, 1655, 1630, 1600, 1225, 1170, 1070, 920, 745, 690	0.89 (3H, t, $J=5$ Hz, CH_2CH_3), 1.20—1.60 (13H, m, $\text{COCH}_2\text{-(CH}_2)_5\text{CH}_3$ and CHCH_3), 2.85 (2H, t, $J=7$ Hz, $\text{COCH}_2\text{-CH}_2$), 3.62 (1H, q, $J=7$ Hz, NCH_2CH_3), 4.11 and 5.10 (each 1H, d, $J=15$ Hz, NCH_2Ar), 7.28 (5H, s, Ar-H), 11.75 (1H, br s, OH)
30	(Film) 1715, 1620 (br), 1225, 1165, 1065, 915	0.89 (3H, t, $J=5$ Hz, CH_2CH_3), 1.28 (14H, br s, $\text{COCH}_2\text{-(CH}_2)_7\text{CH}_3$), 2.85 (2H, t, $J=6$ Hz, COCH_2CH_2), 3.62 (1H, q, $J=7$ Hz, NCH_2CH_3), 4.12 and 5.10 (each 1H, d, $J=15$ Hz, NCH_2Ar), 7.29 (5H, s, Ar-H), 11.15 (1H, br s, OH)
31	(Nujol) 1708, 1623, 1248, 1210, 1065, 975, 950, 890	0.98 (3H, t, $J=6$ Hz, CH_2CH_3), 1.63 (2H, m, $\text{COCH}_2\text{CH}_2\text{-CH}_3$), 2.82 (2H, t, $J=7$ Hz, COCH_2CH_2), 3.72 and 5.30 (each 1H, d, $J=15$ Hz, NCH_2Ar), 4.51 (1H, s, NCHAr), 7.0—7.6 (10H, m, Ar-H), 11.75 (1H, br s, OH)
32	(Nujol) 1710, 1620 (br), 1250, 1170, 950, 880	0.88 (3H, t, $J=5$ Hz, CH_2CH_3), 1.4 (6H, m, $\text{COCH}_2\text{(CH}_2)_3\text{-CH}_3$), 2.82 (2H, t, $J=7$ Hz, COCH_2CH_2), 3.71 and 5.29 (each 1H, d, $J=15$ Hz, NCH_2Ar), 4.50 (1H, s, NCHAr), 7.3 (10H, m, Ar-H), 13.0 (1H, br s, OH)

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus, model MP-S3, and are uncorrected. IR spectra were measured with a Hitachi EPI-S infrared spectrometer and NMR spectra were measured with a Hitachi Perkin-Elmer R-20A spectrometer at 60 MHz using tetramethylsilane (TMS) as an internal standard. Mass spectra (MS) were measured with JEOL D-300 spectrometer.

3-Acetyl-1-benzyl-5-methyltetramic Acid (5)—Diketene (2.83 g, 34 mmol) was added dropwise to ethyl 2-benzylaminopropionate (**1d**)⁹⁾ (5.58 g, 27 mmol) with stirring and cooling (5 °C) under an N_2 atmosphere. When the

TABLE III. Yield and Boiling Point of 4-Substituted Ethyl Acetoacetates 7

$\begin{array}{c} \text{R}^2\text{COCH}_2\text{CO}_2\text{C}_2\text{H}_5 \\ 7 \end{array}$		
R ²	Yield (%)	bp (°C) (Torr)
a (CH ₂) ₂ CH ₃	60	114—118 (27) ¹⁴⁾
b (CH ₂) ₄ CH ₃	44	89—91 (2—3) ¹⁴⁾
c (CH ₂) ₆ CH ₃	61	112—115 (2) ¹⁴⁾
d (CH ₂) ₈ CH ₃	84	125—130 (3) ⁵⁾
e (CH ₂) ₁₀ CH ₃	34	85—95 (0.6) ¹⁵⁾
f (CH ₂) ₁₂ CH ₃	34	147—153 (1) ¹⁶⁾
g CH=CH(CH ₂) ₆ CH ₃	53	119—130 (0.9—1.5) ¹⁷⁾
h (CH ₂) ₃ C ₆ H ₅	89	115—125 (1) ^{a)}
i CH(C ₆ H ₅) ₂	47	162—172 (0.4—0.8) ¹⁸⁾

a) IR $\nu_{\text{max}}^{\text{film}}$ cm⁻¹: 1740, 1715, 1640, 1605, 1500, 1315, 1250, 1180, 1100, 1035, 750, 705. NMR (CDCl₃) δ : 1.26 (3H, t, $J=7$ Hz, CH₂CH₃), 1.6—2.2 (2H, m, ArCH₂CH₂CH₂CO), 2.55 and 2.65 (each 2H, t, $J=6$ Hz, ArCH₂CH₂CH₂CO), 3.40 (2H, s, COCH₂CO₂C₂H₅), 4.19 (2H, q, $J=7$ Hz, OCH₂CH₃), 7.22 (5H, s, Ar-H). Exact mass Calcd for C₁₄H₁₆O₂ (M⁺ - H₂O): 216.1150. Found: 216.1159.

addition was over, the reaction mixture was warmed to room temperature and stirred for 0.5 h. Excess diketene was evaporated off under reduced pressure to leave the *N*-acetoacetyl amino acid ester. A methanolic solution of CH₃ONa [prepared from Na metal (0.75 g, 33 mg atm) and 70 ml of methanol] was added to the product obtained above at 20 °C with stirring. After addition of benzene (40 ml), the reaction mixture was refluxed for 4 h 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 to pH 2 with conc. H₂SO₄ under cooling. The acidic solution was extracted three times with ether and the extracts were washed with saturated brine and dried over Na₂SO₄. The solvent was evaporated off *in vacuo* to give 6.6 g of **5** (quantitative yield) as a viscous oil. An analytical sample was prepared by bulb-to-bulb distillation. bp 144—146 °C (oven temperature) (0.6 Torr). IR $\nu_{\text{max}}^{\text{film}}$ cm⁻¹: 1710, 1630 (br), 1230, 920. NMR (CDCl₃) δ : 1.30 (3H, d, $J=7$ Hz, CHCH₃), 2.45 (3H, s, COCH₃), 3.63 (1H, q, $J=7$ Hz, CHCH₃), 4.13 and 5.14 (each 1H, d, $J=15$ Hz, NCH₂C₆H₅), 7.2—7.4 (5H, m, Ar-H).

Other 3-acetyltetramic acid derivatives (**2**—**4** and **6**) were synthesized by the same procedures from **1a**,¹⁰⁾ **1b**,¹¹⁾ **1c**,¹²⁾ and **1e**,¹³⁾ and their physico-chemical data are listed in Tables I and II.

4-Substituted Ethyl Acetoacetate (7)—The previously reported method for the synthesis of ethyl 3-oxodecanoate (**7d**)⁵⁾ was employed for the preparation of **7**.

1-Benzyl-3-butyryltetramic Acid (23)—A mixture of ethyl benzylaminoacetate (**1c**)¹²⁾ (5.76 g, 30 mmol) and ethyl 3-oxohexanoate (**7a**) (4.56 g, 28.8 mmol) in dry xylene (38 ml) was heated at 125—130 °C with stirring for 20.5 h. The cooled solution was added to methanolic CH₃ONa [prepared from 0.7 g (30.4 mg atm) of Na metal and 31 ml of methanol] at room temperature with stirring. The whole was stirred at room temperature for 43 h. Water was added to the reaction mixture and the organic layer was separated and extracted twice with water. The original water layer and the extracts were combined and acidified to pH 2—3 with 2N HCl under cooling. The acidic solution was extracted three times with chloroform and the extracts were washed with saturated brine, then dried over Na₂SO₄. The solvent was removed under reduced pressure to give 4.885 g (66%) of **23**, which was recrystallized from benzene-hexane to furnish colorless needles. mp 74 °C IR $\nu_{\text{max}}^{\text{Nujol}}$ cm⁻¹: 1720, 1645, 1600. NMR (CDCl₃) δ : 1.00 (3H, t, $J=7.5$ Hz, CH₂CH₃), 1.72 (2H, m, CH₂CH₂CH₃), 2.83 (2H, t, $J=7.5$ Hz, COCH₂CH₂), 3.60 (2H, s, NCH₂C), 4.60 (2H, s, NCH₂-Ar), 7.30 (5H, s, Ar-H), 10.77 (1H, brs, OH).

Other 3-acyltetramic acid derivatives (**9**—**22** and **24**—**32**) were synthesized by the same procedures and their physico-chemical data are listed in Tables I and II.

Antimicrobial Tests—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 *N,N*-dimethylformamide (DMF) and acetone to give various concentrations. The agar was then poured into a Petri dish, and solidified. A loopful of test organism suspension was streaked on the agar plate and the plate was incubated at 33 °C for 18—20 h. The antimicrobial activity was expressed as the minimum inhibitory concentration (MIC, $\mu\text{g/ml}$). The results are summarized in Table I.

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