[Chem. Pharm. Bull. 32(10)4197—4204(1984)]

# Syntheses and Antimicrobial Activities of 3-Acyltetramic Acid Derivatives

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(Received March 21, 1984)

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 subtillis* and *Staphylococcus aureus*.

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

Tenuazoic acid,[1] streptolydigin,[2] tirandamycin[3] 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 $\beta$-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. subtillis* 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 | *B. subtilis* | *St. aureus* |
| **2**[7] | $CH_3$ | | H | Quant. | 51—52 | $C_7H_9NO_3$ | | 155.0561 (155.0582) | | 100 | 100 |
| **9** | $CH_3$ | $(CH_2)_6CH_3$ | H | 49 | 189—191 (0.6) | $C_{13}H_{21}NO_3$ | | 239.1508 (239.1520) | | 50 | 25 |
| **10** | $CH_3$ | $(CH_2)_8CH_3$ | 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** | $CH_3$ | $CH=CH(CH_2)_6CH_3$ | H | 15 | 170—172 (0.6) | $C_{15}H_{23}NO_3$ | | 265.1683 (265.1678) | | 1.56 | 12.5 |
| **14** | $CH_3$ | $(CH_2)_3C_6H_5$ | H | 53 | 194—196 (0.6) | $C_{15}H_{17}NO_3$ | | 259.1224 (259.1208) | | 25 | 50 |
| **15** | $CH_3$ | $CH(C_6H_5)_2$ | 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) | $C_8H_{11}NO_3$ | 56.05 (56.79 | 6.46 6.55 | 8.14 8.28) | 100 | 100 |
| **16** | $CH_3$ | $(CH_2)_4CH_3$ | $CH_3$ | 71 | 118—120 (0.6) | $C_{12}H_{19}NO_3$ | | 225.1390 (225.1365) | | 100 | 50 |
| **17** | $CH_3$ | $(CH_2)_6CH_3$ | $CH_3$ | 40 | 151—154 (0.7) | $C_{14}H_{23}NO_3$ | | 253.1679 (253.1678) | | 12.5 | 12.5 |
| **18** | $CH_3$ | $(CH_2)_8CH_3$ | $CH_3$ | 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** | $CH_3$ | $(CH_2)_3C_6H_5$ | $CH_3$ | 37 | 185—186 (0.6) | $C_{16}H_{19}NO_3$ | | 273.1367 (273.1365) | | 25 | 25 |
| **22** | $CH_3$ | $CH(C_6H_5)_2$ | $CH_3$ | 26 | 113—114 | $C_{20}H_{19}NO_3$ | 74.96 (74.74 | 5.75 5.96 | 4.35 4.36) | 12.5 | 12.5 |
| **4**[7] | $CH_2C_6H_5$ | | H | 26 | 74—76 | $C_{13}H_{13}NO_3$ | 67.31 (67.52 | 5.65 5.67 | 6.02 6.06) | 100 | 100 |
| **23** | $CH_2C_6H_5$ | $(CH_2)_2CH_3$ | H | 66 | 74 | $C_{15}H_{17}NO_3$ | 69.61 (69.48 | 6.59 6.61 | 5.46 5.40) | 25 | 25 |
| **24** | $CH_2C_6H_5$ | $(CH_2)_4CH_3$ | H | 20 | 190—192 (0.7) | $C_{17}H_{21}NO_3$ | | 287.1515 (287.1520) | | 6.25 | 6.25 |
| **25** | $CH_2C_6H_5$ | $(CH_2)_6CH_3$ | H | 71 | 52.5—53 | $C_{19}H_{25}NO_3$ | 72.37 (72.35 | 7.98 7.99 | 4.44 4.44) | 12.5 | 6.25 |
| **26** | $CH_2C_6H_5$ | $(CH_2)_8CH_3$ | H | 29 | 214—216 (0.6) | $C_{21}H_{29}NO_3$ | | 343.2139 (343.2146) | | 50 | 12.5 |
| **5** | $CH_2C_6H_5$ | | $CH_3$ | 52 | 144—146 (0.6) | $C_{14}H_{15}NO_3$ | 68.23 (68.55 | 6.15 6.16 | 5.77 5.71) | 100 | 100 |
| **27** | $CH_2C_6H_5$ | $(CH_2)_2CH_3$ | $CH_3$ | 56 | 160—163 (0.6) | $C_{16}H_{19}NO_3$ | | 273.1365 (273.1365) | | 50 | 50 |
| **28** | $CH_2C_6H_5$ | $(CH_2)_4CH_3$ | $CH_3$ | 35 | 150—153 (0.5) | $C_{18}H_{23}NO_3$ | | 301.1674 (301.1676) | | 50 | 25 |
| **29** | $CH_2C_6H_5$ | $(CH_2)_6CH_3$ | $CH_3$ | 19 | 95—98 | $C_{20}H_{27}NO_3$ | | 329.1962 (329.1990) | | 100 | 6.25 |
| **30** | $CH_2C_6H_5$ | $(CH_2)_8CH_3$ | $CH_3$ | 23 | 164—166 | $C_{22}H_{31}NO_3$ | | 357.2303 (357.2303) | | 50 | 50 |
| **6**[8] | $CH_2C_6H_5$ | | $C_6H_5$ | 50 | 129—130 | $C_{19}H_{17}NO_3$ | 74.05 (74.25 | 5.47 5.58 | 4.54 4.56) | 25 | 50 |
| **31** | $CH_2C_6H_5$ | $(CH_2)_2CH_3$ | $C_6H_5$ | 49 | 115—118 | $C_{21}H_{21}NO_3$ | 75.03 (75.20 | 6.32 6.31 | 4.22 4.18) | 12.5 | 25 |
| **32** | $CH_2C_6H_5$ | $(CH_2)_4CH_3$ | $C_6H_5$ | 28 | 93—94 | $C_{23}H_{25}NO_3$ | 75.82 (76.00 | 6.96 6.93 | 3.76 3.85) | 50 | 50 |

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

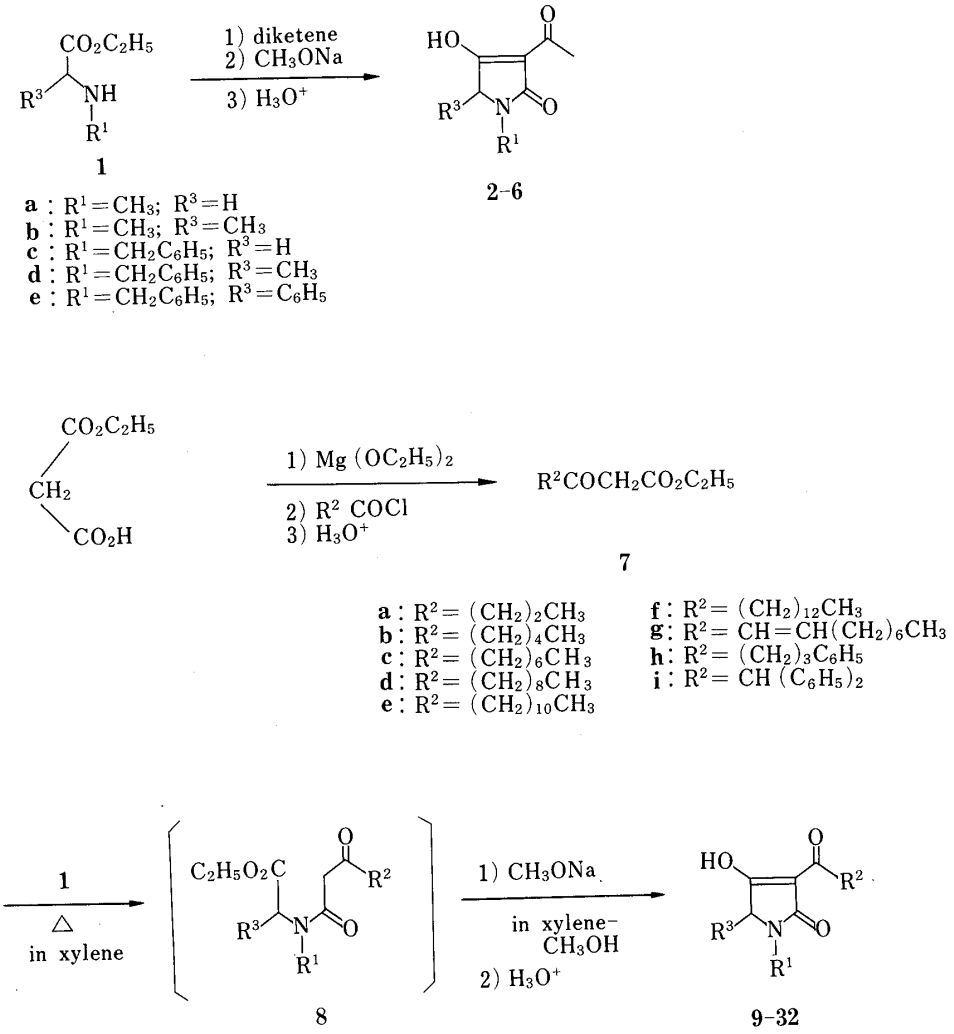


Chart 1

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

In the first series ($R^1=CH_3$ and $R^3=H$) (**2** and **9**—**12**), the highest activity was found with **10** ($R^2=(CH_2)_8CH_3$; molecular weight (MW) 267) and **11** ($R^2=(CH_2)_{10}CH_3$; MW 295); the mean molecular weight of **10** and **11** is 281. In the second series ($R^1=CH_3$ and $R^3=CH_3$) (**3** and **16**—**20**), the highest activity was found with **18** ($R^2=(CH_2)_8CH_3$; MW 281). Compound **24** ($R^1=CH_2C_6H_5$, $R^2=(CH_2)_4CH_3$, and $R^3=H$; MW 287) showed the highest activity in the third series ($R^1=CH_2C_6H_5$, $R^3=H$) (**4** and **23**—**26**). Among **5** and **27**—**30** ($R^1=CH_2C_6H_5$ and $R^3=CH_3$), the highest activity was found with **27** ($R^2=(CH_2)_2CH_3$; MW 273) and **28** ($R^2=(CH_2)_4CH_3$; MW 301) (mean MW of **27** and **28**, 287) against *B. subtillis*, and with compound **29** ($R^2=(CH_2)_6CH_3$; MW 329) against *St. aureus*. In the last series ($R^1=CH_2C_6H_5$ and $R^3=C_6H_5$) (**6**, **31** and **32**), the highest activity was found with **31** ($R^2=(CH_2)_2CH_3$; MW 349) against both microorganisms.

It may be concluded that the antimicrobial activity against *B. subtillis* and *St. aureus* requires a compound with a molecular weight of 280—290 in the case of $R^3=H$ or $CH_3$ 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 ($CDCl_3$) $\delta$: |
|---|---|---|
| **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, $CHC\underline{H}_3$), 2.42 (3H, s, $COCH_3$), 2.98 (3H, s, $NCH_3$), 3.72 (1H, q, $J$=7 Hz, $C\underline{H}CH_3$), 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, $CHC\underline{H}_3$), 2.46 (3H, s, $COCH_3$), 3.63 (1H, q, $J$=7 Hz, $NC\underline{H}CH_3$), 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, NCH(Ar)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, $CH_2C\underline{H}_3$), 1.30 (10H, br s, $COCH_2$-$(C\underline{H}_2)_5CH_3$), 2.84 (2H, t, $J$=7 Hz, $COC\underline{H}_2CH_2$), 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, $CH_2C\underline{H}_3$), 1.30 (14H, br s, $COCH_2$-$(C\underline{H}_2)_7CH_3$), 2.85 (2H, t, $J$=7 Hz, $COC\underline{H}_2CH_2$), 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, $CH_2C\underline{H}_3$), 1.28 (18H, br s, $COCH_2$-$(C\underline{H}_2)_9CH_3$), 2.83 (2H, t, $J$=7 Hz, $COC\underline{H}_2CH_2$), 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, $CH_2C\underline{H}_3$), 1.27 (22H, br s, $COCH_2$-$(C\underline{H}_2)_{11}CH_3$), 2.83 (2H, t, $J$=7 Hz, $COC\underline{H}_2CH_2$), 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, $CH_2C\underline{H}_3$), 1.32 (10H, br s, $=CHCH_2$-$(C\underline{H}_2)_5CH_3$), 2.30 (2H, m, $=CHC\underline{H}_2CH_2$), 3.02 (3H, s, $NCH_3$), 3.72 (2H, s, $NCH_2C$), 7.05—7.30 (2H, m, $COC\underline{H}=C\underline{H}CH_2$), 12.65 (1H, br s, OH) |
| **14** | (Film) 1715, 1655, 1620, 1245, 1165, 985, 910 | 1.98 (2H, m, $COCH_2C\underline{H}_2Ar$), 2.75 (4H, m, $COC\underline{H}_2CH_2C\underline{H}_2Ar$), 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, $COC\underline{H}Ar_2$), 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, $CH_2C\underline{H}_3$), 1.35 (3H, d, $J$=7 Hz, $CHC\underline{H}_3$), 1.2—1.6 (6H, m, $COCH_2(C\underline{H}_2)_3CH_3$), 2.83 (2H, t, $J$=7 Hz, $COC\underline{H}_2CH_2$), 2.98 (3H, s, $NCH_3$), 3.70 (1H, q, $J$=7 Hz, $NC\underline{H}CH_3$), 11.70 (1H, br s, OH) |
| **17** | (Film) 1710, 1660, 1620, 1242, 1170, 1100, 1035, 980, 915 | 0.88 (3H, t, $J$=5 Hz, $CH_2C\underline{H}_3$), 1.35 (3H, d, $J$=6 Hz, $CHC\underline{H}_3$), 1.2—1.5 (10H, m, $COCH_2(C\underline{H}_2)_5CH_3$), 2.83 (2H, t, $J$=7 Hz, $COC\underline{H}_2CH_2$), 2.98 (3H, s, $NCH_3$), 3.70 (1H, q, $J$=6 Hz, $NC\underline{H}CH_3$), 12.03 (1H, br s, OH) |
| **18** | (Film) 1715, 1660, 1625, 1235, 1060, 920, 750 | 0.90 (3H, t, $J$=5 Hz, $CH_2C\underline{H}_3$), 1.27 (14H, br s, $COCH_2$-$(C\underline{H}_2)_7CH_3$), 1.30 (3H, d, $J$=7 Hz, $CHC\underline{H}_3$), 2.83 (2H, t, $J$=7 Hz, $COC\underline{H}_2CH_2$), 2.98 (3H, s, $NCH_3$), 3.70 (1H, q, $J$=7 Hz, $NC\underline{H}CH_3$) |
| **19** | (Film) 1711, 1650, 1623, 1232, 1162, 1060, 920 | 0.89 (3H, t, $J$=5 Hz, $CH_2C\underline{H}_3$), 1.35 (3H, d, $J$=7 Hz, $CHC\underline{H}_3$), 1.27 (18H, br s, $COCH_2(C\underline{H}_2)_9CH_3$), 2.82 (2H, t, $J$=7 Hz, $COC\underline{H}_2CH_2$), 2.97 (3H, s, $NCH_3$), 3.69 (1H, q, $J$=7 Hz, $NC\underline{H}CH_3$), 12.90 (1H, s, OH) |
| **20** | (Film) 1710, 1650, 1615, 1230, 1165, 1060, 920 | 0.89 (3H, t, $J$=5 Hz, $CH_2C\underline{H}_3$), 1.25 (22H, s, $COCH_2$-$(C\underline{H}_2)_{11}CH_3$), 1.35 (3H, d, $J$=7 Hz, $NCHC\underline{H}_3$), 2.81 (2H, t, $J$=7 Hz, $COC\underline{H}_2CH_2$), 2.96 (3H, s, $NCH_3$), 3.68 (1H, q, $J$=7 Hz, $NC\underline{H}CH_3$), 10.30 (1H, br s, OH) |
| **21** | (Film) 1712, 1652, 1725, 1235, | 1.35 (3H, d, $J$=7 Hz, $CHC\underline{H}_3$), 1.8—2.2 (2H, m, $COCH_2C\underline{H}_2$- |

TABLE II. (continued)

| Compd. | IR $\nu_{max}$ cm$^{-1}$: | NMR ($CDCl_3$) $\delta$: |
|---|---|---|
| | 1162, 1065, 920 | $CH_2Ar$), 2.84 (3H, s, $NCH_3$), 3.65 (1H, q, $J$=7 Hz, $NC\underline{H}CH_3$), 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, $CHC\underline{H}_3$), 2.95 (3H, s, $NCH_3$), 3.70 (1H, q, $J$=7 Hz, $NC\underline{H}CH_3$), 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, $CH_2C\underline{H}_3$), 1.5—1.9 (2H, m, $COCH_2$-$C\underline{H}_2CH_3$), 2.83 (2H, t, $J$=7 Hz, $COC\underline{H}_2CH_2$), 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, $CH_2C\underline{H}_3$), 1.4 (6H, br s, $COCH_2$-$(C\underline{H}_2)_3CH_3$), 2.84 (2H, t, $J$=7 Hz, $COC\underline{H}_2CH_2$), 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, $CH_2C\underline{H}_3$), 1.30 (10H, m, $COCH_2$-$(C\underline{H}_2)_5CH_3$), 2.84 (2H, t, $J$=7 Hz, $COC\underline{H}_2CH_2$), 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, $CH_2C\underline{H}_3$), 1.28 (14H, br s, $COCH_2$-$(C\underline{H}_2)_7CH_3$), 2.82 (2H, t, $J$=7 Hz, $COC\underline{H}_2CH_2$), 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, $CH_2C\underline{H}_3$), 1.29 (3H, d, $J$=7 Hz, $NCHC\underline{H}_3$), 1.70 (2H, m, $COCH_2C\underline{H}_2CH_3$), 2.83 (2H, t, $J$=7 Hz, $COC\underline{H}_2CH_2$), 3.62 (1H, q, $J$=7 Hz, $NC\underline{H}CH_3$), 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, $CH_2C\underline{H}_3$), 1.30 (3H, d, $J$=7 Hz, $NCHC\underline{H}_3$), 1.2—1.7 (6H, m, $COCH_2(C\underline{H}_2)_3CH_3$), 2.85 (2H, t, $J$=7 Hz, $COC\underline{H}_2CH_2$), 3.62 (1H, q, $J$=7 Hz, $NC\underline{H}CH_3$), 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, $CH_2C\underline{H}_3$), 1.20—1.60 (13H, m, $COCH_2$-$(C\underline{H}_2)_5CH_3$ and $CHC\underline{H}_3$), 2.85 (2H, t, $J$=7 Hz, $COC\underline{H}_2$-$CH_2$), 3.62 (1H, q, $J$=7 Hz, $NC\underline{H}CH_3$), 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, $CH_2C\underline{H}_3$), 1.28 (14H, br s, $COCH_2$-$(C\underline{H}_2)_7CH_3$), 2.85 (2H, t, $J$=6 Hz, $COC\underline{H}_2CH_2$), 3.62 (1H, q, $J$=7 Hz, $NC\underline{H}CH_3$), 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, $CH_2C\underline{H}_3$), 1.63 (2H, m, $COCH_2C\underline{H}_2$-$CH_3$), 2.82 (2H, t, $J$=7 Hz, $COC\underline{H}_2CH_2$), 3.72 and 5.30 (each 1H, d, $J$=15 Hz, $NC\underline{H}_2Ar$), 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, $CH_2C\underline{H}_3$), 1.4 (6H, m, $COCH_2(C\underline{H}_2)_3$-$CH_3$), 2.82 (2H, t, $J$=7 Hz, $COC\underline{H}_2CH_2$), 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**

$R^2COCH_2CO_2C_2H_5$
**7**

| | $R^2$ | Yield (%) | bp (°C) (Torr) |
|---|---|---|---|
| **a** | $(CH_2)_2CH_3$ | 60 | 114—118 (27)[14] |
| **b** | $(CH_2)_4CH_3$ | 44 | 89—91 (2—3)[14] |
| **c** | $(CH_2)_6CH_3$ | 61 | 112—115 (2)[14] |
| **d** | $(CH_2)_8CH_3$ | 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** | $CH=CH(CH_2)_6CH_3$ | 53 | 119—130 (0.9—1.5)[17] |
| **h** | $(CH_2)_3C_6H_5$ | 89 | 115—125 (1)[a] |
| **i** | $CH(C_6H_5)_2$ | 47 | 162—172 (0.4—0.8)[18] |

*a*) IR $\nu_{max}^{film}$ $cm^{-1}$: 1740, 1715, 1640, 1605, 1500, 1315, 1250, 1180, 1100, 1035, 750, 705. NMR ($CDCl_3$) $\delta$: 1.26 (3H, t, $J=7$ Hz, $CH_2CH_3$), 1.6—2.2 (2H, m, $ArCH_2CH_2CH_2CO$), 2.55 and 2.65 (each 2H, t, $J=6$ Hz, $ArCH_2CH_2CH_2CO$), 3.40 (2H, s, $COCH_2CO_2C_2H_5$), 4.19 (2H, q, $J=7$ Hz, $OCH_2CH_3$), 7.22 (5H, s, Ar-H). Exact mass Calcd for $C_{14}H_{16}O_2$ ($M^+-H_2O$): 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*-acetoacetylamino acid ester. A methanolic solution of $CH_3ONa$ [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. $H_2SO_4$ under cooling. The acidic solution was extracted three times with ether and the extracts were washed with saturated brine and dried over $Na_2SO_4$. 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 $\nu_{max}^{film}$ $cm^{-1}$: 1710, 1630 (br), 1230, 920. NMR ($CDCl_3$) $\delta$: 1.30 (3H, d, $J=7$ Hz, $CHCH_3$), 2.45 (3H, s, $COCH_3$), 3.63 (1H, q, $J=7$ Hz, $CHCH_3$), 4.13 and 5.14 (each 1H, d, $J=15$ Hz, $NCH_2C_6H_5$), 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-oxododecanoate (**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) ws heated at 125—130 °C with stirring for 20.5 h. The cooled solution was added to methanolic $CH_3ONa$ [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 2 N HCl under cooling. The acidic solution was extracted three times with chloroform and the extracts were washed with saturated brine, then dried over $Na_2SO_4$. 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 $\nu_{max}^{Nujol}$ $cm^{-1}$: 1720, 1645, 1600. NMR ($CDCl_3$) $\delta$: 1.00 (3H, t, $J=7.5$ Hz, $CH_2CH_3$), 1.72 (2H, m, $CH_2CH_2CH_3$), 2.83 (2H, t, $J=7.5$ Hz, $COCH_2CH_2$), 3.60 (2H, s, $NCH_2C$), 4.60 (2H, s, $NCH_2$–Ar), 7.30 (5H, s, Ar–H), 10.77 (1H, br s, 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, $\mu$g/ml). The results are summarized in Table I.

**Acknowledgement** The authors are indebted to Dr. K. Konishi and Mr. K. Okamoto, Pesticide Research

Laboratories, Agricultural Chemicals Division, Takeda Chemical Industries Ltd., for carrying out antimicrobial activity tests, and to Mr. M. Kan, Chemical Research Laboratories, Central Research Division, Takeda Chemical Industries Ltd., for microanalysis. Thanks are also due to Mrs. T. Minematsu, Faculty of Pharmaceutical Sciences, Kinki University, for NMR spectral measurement, and to Mr. T. Iwagawa, Faculty of Science, Kagoshima University, for high-resolution mass spectral measurement.

## References and Notes

1) C. E. Stickings, *Biochem. J.*, **72**, 332 (1959); C. E. Stickings and R. J. Townsend, *ibid.*, **78**, 412 (1961).
2) K. L. Rinehart, Jr., J. R. Beck, D. B. Borders, T. H. Kinstle, and D. Krauss, *J. Am. Chem. Soc.*, **85**, 4038 (1963).
3) F. A. MacKellar, M. F. Grostic, E. C. Olson, R. J. Wnuk, A. R. Branfman, and K. L. Rinehart, Jr., *J. Am. Chem. Soc.*, **93**, 4943 (1971).
4) J. L. Van Der Baan, J. W. F. K. Barnick, and F. Bickelhaupt, *Tetrahedron*, **34**, 223 (1978).
5) K. Matsuo, I. Kitaguchi, Y. Takata, and K. Tanaka, *Chem. Pharm. Bull.*, **28**, 2494 (1980).
6) R. N. Lacy, *J. Chem. Soc.*, **1954**, 832, 850.
7) V. J. Lee, A. R. Branfman, T. R. Herrin, and K. L. Rinehart, Jr., *J. Am. Chem. Soc.*, **100**, 4225 (1978).
8) E. A. S. La Croix, S. E. Mhasalkar, P. Mamalis, and F. P. Harrington, *Pestic. Sci.*, **6**, 491 (1975) [*Chem. Abstr.*, **84**, 131414*v* (1976)].
9) J. F. Kerwin, G. C. Hall, F. J. Milnes, I. H. Witt, R. A. McLean, E. Macko, E. J. Fellows, and G. E. Ullyot, *J. Am. Chem. Soc.*, **73**, 4162 (1951).
10) N. Mori, M. Aihara, Y. Asabe, and Y. Tsuzuki, *Bull. Chem. Soc. Jpn.*, **45**, 1786 (1972).
11) H. K. Müller and B. Renk, *Ann.*, **600**, 239 (1956).
12) A. J. Tomisek, *J. Am. Chem. Soc.*, **71**, 1138 (1949).
13) T. A. Martin, W. T. Comer, C. M. Combs, and J. R. Corrigan, *J. Org. Chem.*, **35**, 3814 (1970).
14) Y. Oikawa, K. Sugano, and O. Yonemitsu, *J. Org. Chem.*, **43**, 2087 (1978).
15) Y. Asahina, M. Yanagita, and Y. Sakurai, *Chem. Ber.*, **70B**, 200 (1937).
16) M. Asano and T. Azumi, *Chem. Ber.*, **72B**, 35 (1939).
17) J. A. M. van den Goorbergh and A. van der Gen, *Tetrahedron Lett.*, **21**, 3621 (1980).
18) R. Huisgen, L. A. Feiler, and P. Otto, *Chem. Ber.*, **102**, 3405 (1969).