Bu-2313, A NEW ANTIBIOTIC COMPLEX ACTIVE AGAINST ANAEROBES

III. SEMI-SYNTHESIS OF Bu-2313 A AND B, AND THEIR ANALOGS

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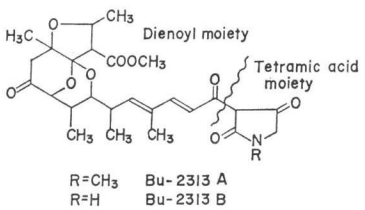
Analogs of Bu-2313 A and B were prepared by C-acylation of tetramic acid derivatives with the dienoic acid moiety obtained by periodate oxidation of Bu-2313 A or B. The C-acylation proceeded in the presence of a strong base such as potassium *t*-butoxide, sodium hydride or lithium hydride, whereas the use of triethylamine afforded O-acylated products. The semi-synthetic Bu-2313 analogs exhibited antibacterial spectra similar to the parent antibiotic but none exceeded Bu-2313 B in activity.

Bu-2313 A and B[1], produced by an oligosporic actinomycete strain, are active against various anaerobic organisms and certain Gram-positive aerobic bacteria. The structures of Bu-2313 A and B (Fig. 1) were determined[2] and shown to be closely related to those of streptolydigin[3] and tirandamycin[4], comprising a dienoyl tetramic acid moiety.

It was found during the course of the structure elucidation of Bu-2313[2] that the periodate oxidation of Bu-2313 A and B yielded the dienoic acid moiety (see Fig. 1), retaining the tricyclic portion intact. The availability of this key carboxylic acid as well as the current interest in acyltetramic acid antibiotics prompted us to attempt to synthesize Bu-2313 analogs having improved properties by acylation of tetramic acids or their equivalents. The present paper described the semi-synthesis of Bu-2313 A and B, and their derivatives. Among the papers[5~10] dealing with synthesis of acyltetramic acids published recently, synthesis of 3-dienoyl tetramic acids was first reported by Lee *et al.*[8], but attempted synthesis of tirandamycin was unsuccessful by their method. Jones *et al.*[10] described synthesis of acyl tetramic acids including a simple dienoyl derivatives, with no application to semi-synthesis of streptolydigin and tirandamycin. The present report, therefore, is the first dealing with 3-dienoyl tetramic acids that possess a naturally occurring complex ring system.

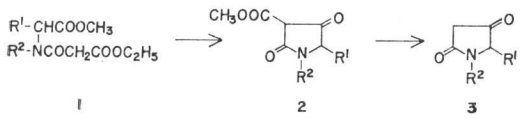
Fig. 1. Structures of Bu-2313 A and Bu-2313 B.



Chemistry

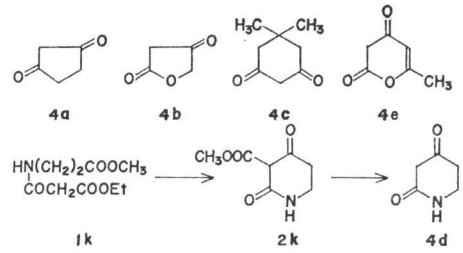
Tetramic acid (**3a**) and its derivatives (**3b**~**3i**) were prepared according to the procedure of Lowe *et al.*[17] as illustrated in Fig. 2. Amino acid esters were acylated with monoethyl malonate using dicyclohexylcarbodiimide (DCC) to give amides (**1a**~**1i**), which cyclized with sodium methoxide affording the 3-methoxycarbonyltetramic acids (**2a**~**2i**). Bhat *et al.*[6] reported the synthesis of 5-ethylidene deriva-

Fig. 2. Preparation of substituted tetramic acids.



	R^1	R^2
a	H	H
b	H	CH_3
c	CH_3	H
d	$CH_3CH=$	H
d′	$CH_3CH(OH)$	H
e	C_2H_5	H
f	$CH_3S(CH_2)_2$	H
g	$CH_3OOC(CH_2)_2$	H
h	$CH_3(CH_2)_5$	H
i	C_6H_5	H
j	$C_6H_5CH=$	H

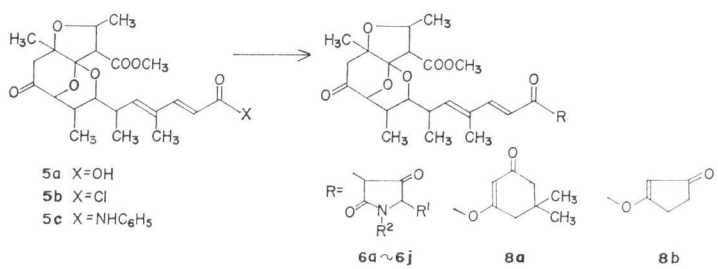
Fig. 3. Cyclic 1,3-diketones used (**4a**～**4e**).



tive (**2d**, ethyl ester) from ethyl N-(ethoxycarbonylacetyl)-α-aminocrotonate. This compound was more conveniently obtained in our laboratory from the threonine derivative (**1d'**) and sodium ethoxide with simultaneous cyclization and dehydration. The 5-benzylidene derivative (**2j**) was prepared from **2a** by condensation with benzaldehyde in the presence of hydrogen chloride[18]. The ester function of **2a**～**2j** was removed by heating in acetonitrile to give the tetramic acids (**3a**～**3j**). The cyclic 1,3-dicarbonyl compounds (Fig. 3) listed below were used as substrates for acylation: 1,3-cyclopentanedione (**4a**); tetronic acid (**4b**)[19], which occurs as a constituent of some fungal metabolites[12,13]; dimedone (**4c**); 2,4-diketopiperidine (**4d**), the 5,6-dehydro derivative of which occurs in mocimycin[14], efrotomycin[15] and aurodox (X-5108)[16]; 4-hydroxy-6-methyl-2-pyrone (**4e**), the 5,6-dihydro derivative of which is a constituent of an antifungal metabolite, alternaric acid[11]. They are commercially available except **4d**, which was prepared from β-alanine methyl ester *via* the malonamide (**1k**) and the cyclic ester (**2k**) in a similar manner to the preparation of tetramic acids.

The dienoic acid (**5a**)[2], prepared by periodate oxidation of Bu-2313 A and Bu-2313 B, was transformed into the acid chloride (**5b**) with thionyl chloride, which was then converted into the anilide (**5c**) to confirm the acid chloride structure (Fig. 4).

Fig. 4. Preparation of Bu-2313 and related compounds.

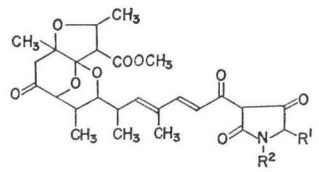


Borontrifluoride etherate[6], triethylamine[7], and thallium salts[8] have been used in acylation of tetramic acids. In our attempts to acylate tetramic acid (**3a**) with the acid chloride (**5b**), the use of borontrifluoride etherate resulted in extensive degradation of **5b** during the reaction. Acylation with **5b** using triethylamine did not give the desired product. Reaction of the acid chloride (**5b**) with dimedone (**4c**), as a simple model for cyclic β-diketones, in the presence of triethylamine did not give any desired C-acyl derivative, but gave the O-acylated derivative (**8a**) in 87% yield. The PMR spectrum showed an olefinic proton signal at δ 5.87 as a broad singlet along with the signals due to the dienoyl

and dimedone moieties. The UV spectrum of compound **8a** did not show the pH-dependent shift, which was reported[7] to be characteristic of the 2-acyl-1,3-dicarbonyl derivatives. Acylation of 1,3-cyclopentanedione (**4a**) in the presence of triethylamine also afforded the O-acylated product **8b** in 51% yield.

The intended C-acylation was successfully achieved by using a stronger base instead of triethylamine. In general, the employment of ordinary strong bases such as potassium *t*-butoxide, sodium hydride and lithium hydride in DMF was found to be effective in C-acylation of the tetramic acid derivatives. Thus, acylation of tetramic acid (**3a**) with **5b** was carried out in a dry DMF solution in the presence of potassium *t*-butoxide (Method A) to give Bu-2313 B in 11% yield, which was identical in all respects with the natural antibiotic. Likewise, Bu-2313 A was prepared semi-synthetically by acyl-

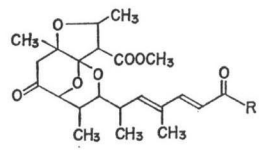
Table 1. Bu-2313 A, Bu-2313 B and 3-acyl-5-substituted tetramic acids.

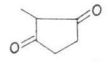
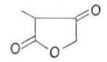
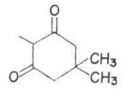
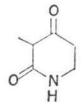
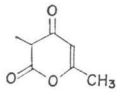


Compound	R^1	R^2	Method	Yield (%)	Mp (°C)	λ_{max} nm (ϵ)	
						Acidic*1	Basic*2
6a	H (Synthetic Bu-2313 B)	H	A	11	130~133	237(10,500) 354(34,300)	255(15,700) 285(18,900) 332(19,900)
6b	H (Synthetic Bu-2313 A)	CH_3	B	6	103~105	242.5(8,900) 357.5(30,900)	264(17,900) 288(17,800) 336(17,400)
6c	CH_3	H	B	50	133~135	236(7,600) 352(33,500)	255(14,200) 283(16,900) 330(18,700)
6d	$CH_3CH=$	H	B	2	120~123	247(14,100) 280(18,600) 356(40,100)	258(21,400) 294(33,000) 346(17,700)
6e	C_2H_5	H	B	34	109~113	238(9,600) 354(34,000)	257(15,000) 285(7,600) 334(19,200)
6f	$CH_3S(CH_2)_2$	H	B	13	137~140	238(8,000) 353(29,400)	258(15,300) 279(17,500) 331(16,000)
6g	$CH_3OOC(CH_2)_2$	H	B	30	132~135	237(7,600) 353(25,900)	260(13,900) 279(17,200) 330(14,000)
6h	$CH_3(CH_2)_5$	H	B	6	103~109	236(8,100) 353(31,200)	256(16,000) 280(18,700) 331(17,500)
6i	C_6H_5	H	B	8	126~129	240(8,200) 355(28,400)	260(14,800) 281(15,500) 333(17,000)
6j	$C_6H_5CH=$	H	B	6	148~153	227(11,100) 360(44,900)	243(14,000) 293(26,800) 333(40,600)

*1: 0.001 N HCl in 99% ethanol. *2: 0.001 N NaOH in 99% ethanol.

Table 2. 2-C-Acylated cyclic 1,3-dicarbonyl derivatives.



Compound	R	Method	Yield (%)	Mp (°C)	λ_{max} nm (ϵ)	
					Acidic*1	Basic*2
7a		B	4	103~106	228(11,900) 357(24,000)	248(22,000) 292(17,400) 322(17,900)
7b		B	16	93~97	232 (6,000) 350(22,600)	239(14,800) 313(16,800)
7c		C	20	68~70	251(13,000) 346(21,200)	271(31,300) 328 (7,900)
7d		C	13	103~106	246 (9,900) 346(29,400)	264(23,700) 333(14,900)
7e		D	5	98~108	241(10,500) 353(24,600)	270(17,300) 330(11,200)

*1: 0.001 N HCl in 99% ethanol. *2: 0.001 N NaOH in 99% ethanol.

ation of N-methyltetramic acid (**3b**) with **5b** in DMF in the presence of sodium hydride (Method B). Most of the C-acylated products in the present study (**6b**~**6j**, **7a** and **7b**) were prepared by Method B. Lithium hydride was effective in the acylation of dimedone (**4c**) and 2,4-diketopiperidine (**4d**) with **5b** in dry DMF (Method C). Acylation of 4-hydroxy-6-methyl-2-pyrone (**4e**) was accomplished by heating a mixture of **4e**, the carboxylic acid **5a** and phosphorus pentoxide in benzene (Method D) to give the desired product **7e** in 5% yield, whereas Method B gave only a trace of **7e**. Tables 1 and 2 show the results of C-acylation of the tetramic acids (**3a**~**3j**) and the cyclic 1,3-dicarbonyls (**4a**~**4e**), respectively.

As indicated in Tables 1 and 2, the C-acylated products, **6a**~**6j** and **7a**~**7e**, were characterized by a bathochromic shift of the maximum at the shortest wavelength (near 240 nm) in the UV spectrum and a hypsochromic shift of the maximum at longest wavelength (near 350 nm) on changing from acidic to basic solution[2,8]. The magnitude of the shifts is dependent upon the β-tricarbonyl moieties involved. Bathochromic shifts of 18~23 nm and hypsochromic shifts of 20~23 nm were observed in all of the 3-acyltetramic acids except **6d** and **6j**, both of which had an additional methylidene group conjugated to the β-triketone chromophore. The structures of the C-acylated derivatives were also supported by their PMR spectra, showing protons of the dienoyl part at similar chemical shifts to those in the original antibiotics[2], together with distinguishable peaks depending on the β-tricarbonyl moieties involved.

Antibacterial Activity

Tables 3 shows *in vitro* activity of synthetic Bu-2313 A and B, and their analogs against two anaerobes (*Bacteroides fragilis* A20926 and *Propionibacterium acnes* A21933) and an aerobe (*Streptococcus pyogenes* A 9604). The data presented are representative of those obtained from a larger group of 12 anaerobic and 32 aerobic test organisms that were used in our primary screen for evaluation of this series of compounds. All of the compounds prepared in the present study showed an antibacterial spectrum similar to that of the parent antibiotics. The synthetic Bu-2313 A and B are as active as the natural antibiotics. In 3-acyl-tetramic acids (**6a**~**6j**), an increase in the chain length of the 5'-substituent led to a diminution in activity, especially against *S. pyogenes*. The 5'-methyl derivative (**6c**) is the most active among the semi-synthetic analogs against the 3 strains listed in Table 3, being more active than Bu-2313 A against *B. fragilis* and *S. pyogenes*, but only half as active as Bu-2313 B. The 5'-ethyl derivative (**6e**) showed activity comparable to Bu-2313 A.

Table 3. *In vitro* activity of synthetic Bu-2313 and analogs.

Compound	MIC (mcg/ml)		
	B. fragilis A20926	*P. acnes* A21933	*S. pyogenes* A9604
6a synthetic Bu-2313 B	0.05	0.2	0.8
6b synthetic Bu-2313 A	0.4	0.4	3.1
6c	0.1	0.4	1.6
6d	6.3	0.8	12.5
6e	0.1	0.8	3.1
6f	0.2	0.8	6.3
6g	0.4	1.6	12.5
6h	1.6	3.1	100
6i	1.6	1.6	1.6
6j	0.1	1.6	25
7a	0.1	0.4	6.3
7b	25	6.3	12.5
7c	6.3	1.6	25
7d	3.1	0.8	3.1
7e	12.5	3.1	25
Bu-2313 A	0.2	0.2	6.3
Bu-2313 B	0.05	0.2	0.8

A similar antibacterial spectrum was shown by 2-acyl-1,3-dicarbonyl derivatives (**7a**~**7e**) possessing ring systems different from the naturally occurring tetramic acid. The 1,3-cyclopentanedione derivative (**7a**), the most active member of this series, showed better anti-*Bacteroides* activity than Bu-2313 A, with the same degree of activity against *P. acnes* and *S. pyogenes*.

Experimental

3-Alkoxycarbonyl-2,4-diketopyrrolidines (**2a**~**2i**) and 3-methoxycarbonyl-2,4-diketopiperidine (**2k**)

Amino acid esters, prepared from amino acids and alcohol by the action of thionyl chloride, was acylated with monoethyl malonate and DCC in methylene chloride by the procedure of LOWE *et al.*[17] giving the requisite malonamide esters (**1a**~**1i** and **1k**) in almost quantitative yields. Without further purification these amides were cyclized by heating with sodium alkoxide in benzene to give 5-membered (**2a**~**2i**) and 6-membered (**2k**) derivatives, as exemplified by the following preparation of **2e** and **2k**.

Preparation of **2e**: To a stirred mixture of 3.91 g (25.5 m moles) of α-aminobutyric acid methyl ester hydrochloride prepared from the amino acid and methanol by the action of thionyl chloride, 2.57 g (25.5 m moles) of triethylamine and 3.62 g (27.4 m moles) of monoethyl malonate in 50 ml of methylene chloride was added 5.25 g (25.5 m moles) of DCC. The mixture was stirred overnight at room temperature and then filtered to remove precipitated dicyclohexylurea. The filtrate was washed with water, dried with sodium sulfate and evaporated to give oily **1e** quantitatively. IR (Film): 3350, 1735, 1645, 1540, 1365, 1200, 1140, 1025 cm^{-1}.

A solution of 6.30 g (25 m moles) of **1e** in 140 ml of dry benzene was heated at reflux for 6 hours under nitrogen with sodium methoxide (prepared from 636 mg (27.6 m moles) of metallic sodium and 22 ml dry methanol). After cooling *ca* 50 ml of water was added to the reaction mixture. The separated water layer was adjusted to pH 2~3 with conc.HCl and extracted with 500 ml of $CHCl_3$. The $CHCl_3$ extracts were dried with Na_2SO_4 and evaporated to dryness *in vacuo*. The residue was triturated with EtOAc to give 3.08 g (65%) of **2e**, mp 182~183°C. IR (KBr): 3190, 1690, 1645, 1610, 1450, 1320, 1180, 1105, 790 cm^{-1}. UV: λ_{max}^{EtOH} 228 nm (ϵ 14,000), 263 nm (ϵ 11,300).

Preparation of **2k**: DCC (1.64 g, 8 m moles) was added to a stirred solution of 1.2 g (8.5 m moles) of β-alanine methyl ester, 1.2 g (8.7 m moles) of monoethyl malonate and 1.1 ml (11.2 m moles) of triethylamine in 20 ml of methylene chloride. The mixture was stirred overnight at 4°C and filtered. The filtrate was washed with water, dried with Na_2SO_4 and evaporated *in vacuo* to give 1.3 g (72%) of **1k**. IR (Film): 3300, 1725, 1650, 1195, 1020 cm^{-1}.

A solution of 1.6 g (7.2 m moles) of **1k** in 35 ml dry benzene was refluxed for 6 hours with stirring under a nitrogen atmosphere with sodium methoxide prepared from 166 mg (7.2 m moles) of metallic sodium and 6 ml of methanol. The mixture was diluted with water under cooling. The aqueous layer was separated, acidified with conc. HCl and shaken with $CHCl_3$. The $CHCl_3$ layer was dried on anhydrous Na_2SO_4 and evaporated to dryness *in vacuo*. The residue was crystallized from methanol to give 693 mg (57%) of **2k**, mp 137~138°C. IR (KBr): 1650, 1570, 1425, 1390, 1240, 1140, 960, 920, 780 cm^{-1}. NMR (DMSO-d_6): δ (ppm) 2.65 (2H, t, J=7 Hz), 3.40 (2H, t, J=7 Hz), 3.86 (3H, s), 6.74 (1H, br), 11.15 (1H, br).

3-Ethoxycarbonyl-5-ethylidene-2,4-diketopyrrolidine (**2d**)

A solution of 2.2 g (9 m moles) of **1d'** in 20 ml of dry benzene was heated at reflux for 2 hours under nitrogen gas with sodium ethoxide prepared from 480 mg (10 m moles) of sodium hydride. After cooling water was added to the reaction mixture with shaking. The water layer was adjusted to pH 1~2 with conc. HCl and extracted with $CHCl_3$ several times. The $CHCl_3$ extracts were dried with Na_2SO_4 and evaporated to dryness *in vacuo*. The residue was triturated with diethyl ether to give 881 mg (50%) of **2d**, mp 181~183°C. IR (KBr): 3170, 1720, 1660, 1605, 1455, 1340, 1190, 1130, 1040, 790 cm^{-1}. NMR (DMSO-d_6): δ (ppm) 1.25 (3H, t, J=7 Hz), 1.82 (3H, d, J=7.5 Hz), 3.67 (1H, s), 4.17 (2H, q, J=7 Hz), 5.62 (1H, q, J=7.5 Hz), 9.53 (1H, br, s).

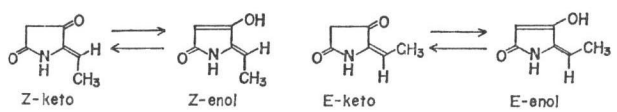
5-Benzylidene-3-methoxycarbonyltetramic acid (**2j**)

3-Methoxycarbonyltetramic acid (**2a**) was condensed with benzaldehyde according to a published procedure[18]. A mixture of 785 mg (5 m moles) of **2a** and 530 mg (5 m moles) of benzaldehyde in 25 ml of dry ethanol was saturated with hydrogen chloride gas. The mixture was stirred at room temperature for 2 days. The separated product was collected by filtration and washed with ethanol to give 232 mg (19%) of **2j**, mp 182.5~183°C.

Tetramic acids (**3a~3j**) and 2,4-diketopiperidine (**4d**)

The alkoxycarbonyl derivatives (**2a~2k**) were heated in acetonitrile for about 2~4 hours to give the dealkoxycarbonyl derivatives (**3a~3j** and **4d**) as shown in the following examples.

Preparation of **3d**: A suspension of 197 mg of **2d** in 20 ml of acetonitrile was heated under reflux for about 2 hours under nitrogen gas to give a solution. The solution was evaporated to dryness *in vacuo* to give 125 mg of practically pure **3d**, which was used as a substrate of C-acylation without purification, mp 178~179°C. IR (KBr): 3150, 2830, 1750, 1710, 1660, 1375, 1355, 1320, 1240, 1060 cm^{-1}. NMR (DMSO-d_6): δ (ppm) 1.71, 1.74 (3H, d, J=7.5 Hz, =CHCH_3), 3.07 (2H×1/2, s, CH_2), 4.79, 4.81 (1H×1/2, s, H〉=〈OH), 5.25, 5.36 (1H, q, J=7.5 Hz, =CHCH$_3$), 8.98, 9.42 (1H, br, 〉NH), 10.0 (1H×1/2, s, H〉=〈OH). The NMR spectrum suggests that this compound exist as a mixture*

* 

of keto-enol tautomers (1: 1) of geometrical isomers (Z: E=1: 1).

Preparation of **4d**: A suspension of 171 mg of **2k** in 50 ml of acetonitrile was refluxed under an atmosphere of nitrogen until a clear solution was attained. The solution was stripped under vacuum to give a quantitative yield of **4d**, mp 88~92°C. IR (KBr): 1725, 1660, 1490, 1460, 1355, 1230, 1100 cm^{-1}.

The dienoic acid chloride (**5b**)

A mixture of 422 mg (1 m mole) of the dienoic acid (**5a**)[2] and 0.8 ml of thionyl chloride in 10 ml of dry methylene chloride was refluxed for 3 hours. The solvent and excess reagent were evaporated *in vacuo*. The oily residue was co-evaporated with three 2-ml portions of dry benzene to give a semi-solid, which was used for C-acylation without further purification.

The dienoic acid anilide (**5c**)

The acid chloride (**5b**), freshly prepared from 30 mg (0.07 m mole) of the carboxylic acid (**5a**) by the preceding procedure, was allowed to react with 2 drops of aniline in 2 ml of dry methylene chloride at room temperature for an hour. The reaction mixture was evaporated *in vacuo*. The solid residue was triturated alternately with 1 N HCl and aq.$NaHCO_3$, filtered, washed with water and dried to give 21 mg (59%) of the anilide (**5c**). Crystallization from ethanol offered 13.5 mg (38%) of colorless needles for analysis. Mp 117~119°C. IR (KBr): 1730, 1660, 1540, 1445, 1205, 755, 690 cm^{-1}. λ_{max}^{EtOH} 278 nm (ε 27,600). NMR ($CDCl_3$): δ (ppm) 0.84 (3H, d, J=7.5 Hz, 8-CH_3), 1.02 (3H, d, J=7 Hz, 6-CH_3), 1.32 (3H, d, J=6 Hz, 15-CH_3), 1.43 (3H, s, 12-CH_3), 1.80 (3H, s, 4-CH_3), 2.56 & 2.96 (2H, ABq, J=17 Hz, 11-CH_2), 2.93 (1H, d, J=9 Hz, 14-H), 3.35 (1H, dd, J=11 & 2 Hz, 7-H), 3.77 (3H, s, $COOCH_3$), 4.01 (1H, d, J=5.5 Hz, 9-H), 4.50 (1H, m, 15-H), 5.88 (1H, d, J=10 Hz, 5-H), 5.91 (1H, d, J=14 Hz, 2-H), 7.30 (6H, m, C_6H_5 & 3-H). Anal. Calcd. for $C_{28}H_{35}NO_7 \cdot \frac{1}{4}H_2O$: C, 66.98; H, 7.13; N, 2.79. Found: C, 66.82; H, 6.98; N, 2.60.

C-Acylation of cyclic 1,3-dicarbonyl compounds

C-Acylation tetramic acid derivatives (**3a**~**3j**) and other cyclic 1,3-dicarbonyl compounds (**4a**~**4e**) with the acid chloride (**5b**) was performed by either one of the following four methods. Results are shown in Tables 1 and 2. Microanalyses (C, H, N) of the products were all coincident with the calculated value within ±0.4% deviation and the mass spectra gave the molecular ions corresponding to the expected structures.

Synthesis of Bu-2313 B (Method A): To a stirred solution of 99 mg (1 m mole) of tetramic acid (**3a**) in 15 ml of dry DMF under nitrogen was added 124 mg of potassium *t*-butoxide under cooling at 0°C. After 30 minutes the acid chloride (**5b**), freshly prepared from 420 mg (1 m mole) of the carboxylic acid (**5a**), in 7 ml of dry DMF was added to the stirred solution. The mixture was stirred for 2 days at room temperature under nitrogen. The reaction mixture was treated with water, adjusted to pH 3 with dil.HCl and shaken with benzene. The benzene layer was washed with saturated brine, dried over Na_2SO_4 and evaporated *in vacuo* to leave an oil. Chromatographic separation of the oil on silica gel, which was pre-treated with triethylamine, using chloroform - ethanol (100: 3) gave 55 mg (11%) of Bu-2313 B (**6a**), which was identical in all respects with the natural antibiotic. Crystallization from petroleum ether-methylene chloride gave pale yellow needles.

Synthesis of Bu-2313 A (Method B): The synthesis of Bu-2313 A (**6b**) is given as a typical example of this category. To a stirred solution of 112 mg (1 m mole) of **3b** and 50 mg (1 m mole) of 50% sodium hydride in 5 ml of dry DMF was added at −25°C under nitrogen the acid chloride (**5b**) prepared from 300 mg (0.71 m mole) of **5a**. The cooling bath was removed and temperature of the reaction mixture was allowed to rise. The mixture was continued to stir overnight at room temperature and then evaporated to dryness *in vacuo*. The resulting oil was treated with benzene and dil.HCl with shaking. The organic layer was washed with water, dried with sodium sulfate and evaporated to dryness to leave 325 mg of solid, which was chromatographed on silica gel (20 g, pre-treated with triethylamine) with CH_2Cl_2 - EtOH (100: 1~2) as eluant to give 91 mg of the crude product. Crystallization from methanol afforded 15 mg (6%) of yellowish needles of Bu-2313 A (**6b**), which was identical with the authentic sample.

Synthesis of **7c** (Method C): The following example illustrates the Method C. A mixture of 47 mg (0.34 m mole) of dimedone (**4c**) and 8 mg (1 m mole) of lithium hydride in 3 ml of dry DMF was

stirred for 4 hours at room temperature. To the solution was added the acid chloride (**5b**) prepared from 100 mg (0.24 m mole) of **5a**. The mixture was stirred for 3.5 days at room temperature under nitrogen and then dried up *in vacuo*. The resulting oil was treated with benzene and dil.HCl. The benzene layer was washed with water, dried with sodium sulfate and evaporated *in vacuo*. The residue was subjected to column chromatography on silica gel (10 ml) with benzene-EtOAc as eluant to give 26 mg (20%) of **7c**.

Synthesis of **7e** (Method D): A mixture of 126 mg (1 m mole) of 4-hydroxy-6-methyl-2-pyrone (**4e**), 422 mg (1 m mole) of the carboxylic acid (**5a**) and 1 g of phosphorus pentoxide in benzene was heated at reflux for 20 hours. The reaction mixture was cautiously treated with ice-water under cooling and shaking. The organic layer was washed with water, dried with Na_2SO_4 and evaporated *in vacuo*. The residue was subjected to chromatography on a silica gel column (pre-treated with triethylamine) giving 27 mg (5%) of **7e**.

The O-acylated derivative of dimedone (**8a**)

To a stirred solution of 17 mg (0.12 m mole) of dimedone (**4c**) and 20 mg (0.19 m mole) of triethylamine in 3 ml of dry methylene chloride was added a solution of the acid chloride (**5b**) in 1 ml of methylene chloride freshly prepared from 50 mg (0.12 m mole) of the dienoic acid (**5a**). The mixture was heated under reflux for 30 minutes, allowed to stand overnight at room temperature under nitrogen, then treated with water and acidified with dil.HCl under shaking. The organic layer was washed with water, dried over Na_2SO_4 and dried up *in vacuo*. The residue was chromatographed on silica gel to give 58 mg of crude **8a**. Crystallization from methanol afforded 54 mg (87%) of pure **8a**, needles, mp 150~151°C. IR (KBr): 1730, 1670, 1620, 1435, 1385, 1310, 1280, 1205, 1130, 1115, 840 cm^{-1}. Anal. Calcd. for $C_{30}H_{40}O_9 \cdot \frac{1}{4}H_2O$: C, 65.62; H, 7.43. Found: C, 65.76; H, 7.36.

The O-acylated product of 1,3-cyclopentanedione (**8b**)

To a stirred solution of 25 mg (0.27 m mole) of 1,3-cyclopentanedione (**4a**) and 40 mg (0.4 m mole) of triethylamine in 1 ml of dry methylene chloride was added under cooling a solution of the acid chloride (**5b**) in 2 ml of dry methylene chloride prepared from 100 mg (0.24 m mole) of the carboxylic acid (**5a**). The mixture was stirred for 3 hours at room temperature, allowed to stand overnight, treated with water and acidified with dil.HCl under stirring. The organic layer was washed with water, dried over Na_2SO_4 and evaporated *in vacuo*. The residue was crystallized from methanol to give 61 mg (53%) of **8b**, mp 187~189°C. Anal. Calcd. for $C_{27}H_{34}O_9 \cdot \frac{1}{2}H_2O$: C, 63.39; H, 6.90. Found: C, 63.28; H, 7.07.

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References

1) TSUKIURA, H.; K. TOMITA, M. HANADA, S. KOBARU, M. TSUNAKAWA, K. FUJISAWA & H. KAWAGUCHI: Bu-2313, a new antibiotic complex active against anaerobes. I. Production, isolation and properties of Bu-2313 A and B. J. Antibiotics 33: 157~165, 1980
2) TSUNAKAWA, M.; S. TODA, T. OKITA, M. HANADA, S. NAKAGAWA, H. TSUKIURA, T. NAITO & H. KAWAGUCHI: Bu-2313, a new antibiotic complex active against anaerobes. II. Structure determination of Bu-2313 A and B. J. Antibiotics 33: 166~172, 1980
3) RINEHART, Jr., K. L.; J. R. BECK, D. B. BORDERS, T. H. KINSTLE & D. KRAUSS: Streptolydigin. III. Chromophore and structure. J. Am. Chem. Soc. 85: 4038~4039, 1963
4) MACKELLAR, F. A.; M. F. GROSTIC, E. C. OLSON, R. J. WNUK, A. R. BRANFMAN & K. L. RINEHART, Jr.: Tirandamycin. I. Structure assignment. J. Am. Chem. Soc. 93: 4943~4945, 1971
5) HARRIS, S. A.; L. V. FISHER & K. FOLKERS: The synthesis of tenuazonic and congeneric tetramic acids. J. Med. Chem. 8: 478~482, 1965
6) BHAT, S. V.; H. KOHL, B. N. GANGULI & N. J. DE SOUZA: Magnesidin-related tetramic acids: Synthesis and structural requirements for antibacterial activity. Eur. J. Med. Chem. 12: 53~57, 1977

7) VAN DER BAAN, J. L.; J. W. F. K. BARNICK & F. BICKELHAUPT: The total synthesis of the antibiotic malonomicin (K 16). Tetrahedron 34: 223~231, 1978
8) LEE, V. J.; R. BRANFMAN, T. R. HERRIN & K. L. RINEHART, Jr.: Synthesis of 3-dienoyl tetramic acids related to streptolydigin and tirandamycin. J. Am. Chem. Soc. 100: 4225~4236, 1978
9) CARTWRIGHT, D.; V. J. LEE & K. L. RINEHART, Jr.: Synthesis of 3-acyl tetramic acids *via* aspartimide rearrangement. J. Am. Chem. Soc. 100: 4237~4239, 1978
10) JONES, R. C. F. & S. SUMARIA: A synthesis of 3-acyl-5-alkyl tetramic acids. Tetrahed. Lett. 1978: 3173~3176, 1978
11) BARTELS-KEITH, J. R.: Alternaric acid. III. Structure. J. Chem. Soc. 1960: 1662~1665, 1960
12) CLUTTERBUCK, P. W.; H. RAISTRICK & F. REUTER: Biochemistry of microorganisms. XLI. The metabolic products of *Penicillium charlisii* G. Smith. 2. The molecular constitution of carolic and carolinic acids. Biochem. J. 29: 300~321, 1935
13) CLUTTERBUCK, P. W.; H. RAISTRICK & F. REUTER: Biochemistry of microorganisms. XLIII. The metabolic products of *Penicillium charlisii* G. Smith. 3. The molecular constitution of carlic and carlosic acids. Biochem. J. 29: 871~883, 1935
14) VOS, C. & P. E. J. VERWIEL: The total structure of the novel antibiotic mocimycin (MYC 8003). Tetrahed. Lett. 1973: 5173~5176, 1973
15) WAX, R.; W. MAIESE, R. WESTON & J. BIRNBAUM: Efrotomycin, a new antibiotic from *Streptomyces lactamdurans*. J. Antibiotics 29: 670~673, 1976
16) MAEHR, H.; M. LEACH, L. YARMCHUK & A. STEMPEL: Antibiotic X-5108. V. Structures of antibiotic X-5108 and mocimycin. J. Am. Chem. Soc. 95: 8449~8450, 1973
17) LOWE, G. & H. W. YEUNG: Synthesis of a β-lactam related to the cephalosporins. J. Chem. Soc. Perkin I 1973: 2907~2910, 1973
18) MULHOLLAND, T. P. C.; R. FOSTER & D. B. HAYDOCK: Synthesis of pyrrolidine-2,4-diones (tetramic acids) and some derivatives. J. Chem. Soc. Perkin I 1972: 2121~2128, 1972
19) ZIMMER, H.; W. W. HILLSTROM, J. C. SCHMIDT, P. D. SEEMUTH & R. VÖGELI: Substituted γ-lactones. 28. 3-(Phenylmethylene)-2,4(3H,5H)-furanodiones. J. Org. Chem. 43: 1541~1544, 1978
20) ISOWA, Y. & M. OHTA: Synthesis of tetramic acids. Bull. Chem. Soc. Jpn. 35: 1941~1943, 1962