STRUCTURES OF BU-2313 A AND B, NEW ANTI-ANAEROBIC ANTIBIOTICS AND SYNTHESES OF THEIR ANALOGS

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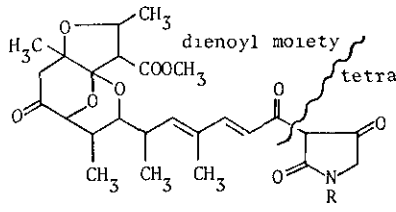
Dedicated to Professor Hamao Umezawa on the occasion of his 65th birthday

Abstract —— An oligosporic actinomycete strain, No. E864-861, produced two new antibiotics, Bu-2313 A ($C_{27}H_{35}NO_9$) and Bu-2313 B ($C_{26}H_{33}NO_9$). Both Bu-2313 A and B exhibited a broad antimicrobial spectrum against gram-positive and gram-negative anaerobic bacteria, and also inhibited the growth of some aerobic bacteria such as streptococci. The structures of Bu-2313 A and B have been elucidated. They belong to the family of antibiotics classified as dienoyltetramic acids. Semi-synthesis of Bu-2313 A and B was performed by C-acylation of tetramic acids with the dienoic moiety obtained by periodate oxidation of Bu-2313. In a similar manner Bu-2313 analogs were prepared by using a variety of tetramic acids and cyclic 1,3-diketones.

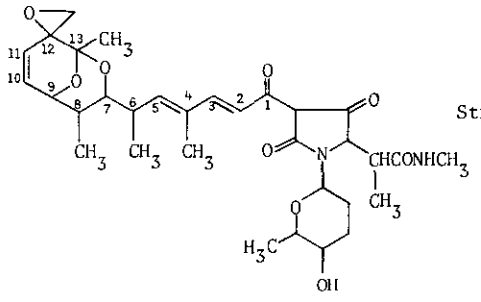
Anaerobic bacteria are involved in a wide variety of human infections, particularly intraabdominal and pelvic infections associated with abscess formation.[1] With the increasing awareness of the role of anaerobic infections in clinical medicine, effective and nontoxic agents active against anaerobic organisms are needed. Benzylpenicillin is considered to be the drug of choice for many anaerobic infections[2] except for those caused by *Bacteroides fragilis*, the single most common anaerobic species found in clinical specimens.[3] As compared with other anaerobes, members of this species are relatively resistant to many antibiotics.

In our antibiotic screening program using *B. fragilis* as one of the assay test organisms, an oligosporic actinomycete strain was found to produce a new antibiotic complex designated as Bu-2313.[4] It was extracted from the fermentation broth and separated into two components A and B. Both components showed antibiotic activity against a variety of anaerobic bacteria as well as some aerobic micro-organisms. The structures of Bu-2313 A and B have been determined,[5] indicating that they belong to the dienoyltetramic acid group of antibiotics which includes streptolydigin[6] and tirandamycin.[7] The present paper describes the structure determination of Bu-2313 A and B, and the semi-synthetic preparation of Bu-2313 A and B as well as analogs of those compounds.

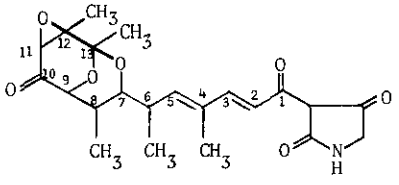


R = CH_3 Bu-2313 A

R = H Bu-2313 B



Streptolydigin



Tirandamycin

I Structure determination

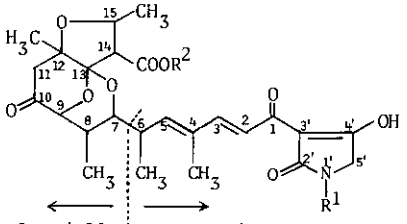
Bu-2313 A(1) and B(2) were isolated from the fermentation broth of an oligosporic actinomycete strain No. E864-861[4] by solvent extraction and column chromatography on Diaion HP-10 (porous polymer resin). Both antibiotics are acidic substances obtained as pale yellow crystals, giving a positive reaction with ferric chloride. Their physico-chemical properties are summarized in Table 1. The molecular formulae of $C_{27}H_{35}NO_9$ and $C_{26}H_{33}NO_9$ were assigned for 1 and 2, respectively, based on their micro-analyses and mass spectra.

The UV spectrum of 1 in acidic solution exhibited absorption maxima at 242, 258, 375 nm ($E^{1\%}_{1cm}$ 170, 645, 555), and that of 2 at 237, 353, 370 nm ($E^{1\%}_{1cm}$ 170, 668, 580). In alkaline solution maxima occurred at 262, 286, 337 nm ($E^{1\%}_{1cm}$ 375, 393, 430) in 1 and at 235, 286, 331 nm ($E^{1\%}_{1cm}$ 345, 411, 467) in 2. These UV absorptions with pH-dependent shifts observed for 1 and 2 are similar to those reported for tirandamycin[8] and streptolydigin,[9] suggesting that Bu-2313 A and B are α,β;γ,δ-dienoyltetramic acid antibiotics.

Table 1 The physico-chemical properties of Bu-2313 A and B

	Bu-2313 A(1)	Bu-2313 B(2)
M.p. (°C)	116 - 118	160 - 162
$[\alpha]_D^{26}$ in MeOH	-58°	-69.9°
Pka' in aq. EtOH	5.2	4.9
Titration Equivalent	519	509
Mass, M^+ (m/e)	517	503

The NMR spectrum of 1 showed three >CH-$\underline{CH_3}$, two >C-$\underline{CH_3}$, one N-$\underline{CH_3}$, one N-$\underline{CH_2}$ and one COO$\underline{CH_3}$ signals, while 2 exhibited three >CH-$\underline{CH_3}$, two >C-$\underline{CH_3}$, one N-$\underline{CH_2}$ and one COO$\underline{CH_3}$ (the latter two signals appeared as a 5H singlet combined), but no N-CH_3 signal. The NMR spectrum of 2 showed an amide NH at δ 6.98 which disappeared by D_2O addition. This NH signal was not present in the spectrum of 1. Similarity of the NMR spectrum of 1 to that of 2 except for the N-CH_3 signal, coupled with molecular ions in their mass spectra (m/e 517 for 1 and m/e 503 for 2), indicated that 1 is an N-methyl derivative of 2. The presence of a methyl ester group was confirmed by hydrolysis of 2 with N-NaOH-MeOH (1:1) at room temperature to afford the acid 3 (mp 223-224 °C, $C_{25}H_{31}NO_9$, M^+ m/e 489) whose NMR spectrum showed no O-methyl signal at around δ 3.76.



1 & 2 m/e283 | 1 m/e235
3 m/e269 | 2 & 3 m/e221

1 R^1 & R^2 = CH_3 Bu-2313A M^+ m/e517
2 R^1 = H R^2 = CH_3 Bu-2313B M^+ m/e503
3 R^1 & R^2 = H M^+ m/e489

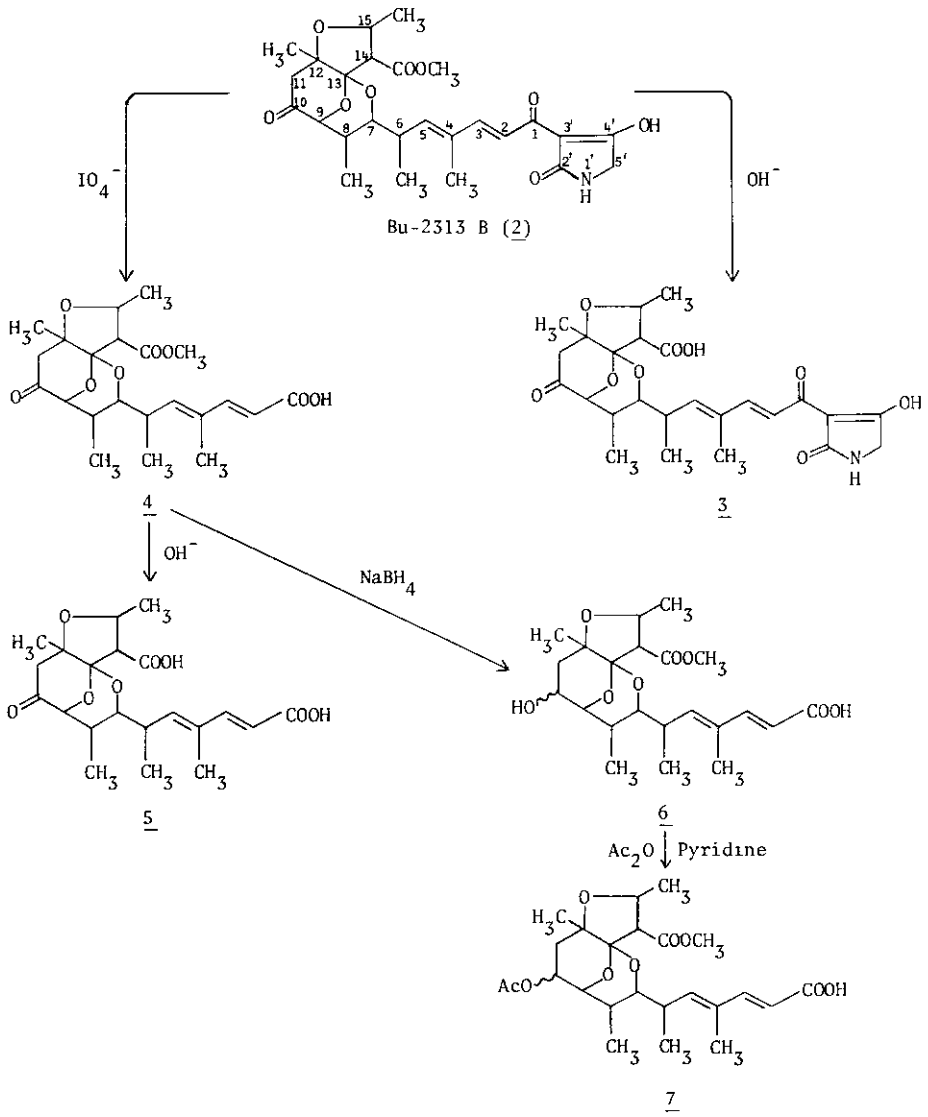
The mass spectra of 1, 2 and 3 gave their molecular ions as well as two intense peaks due to fragmentation corresponding to that in tirandamycin, which reportedly to occurred between C-6 and C-7 of the dienoyl moiety. The mass spectrum of 2 showed the molecular ion peak at m/e 503 along with as intense peak at m/e 283 and a base ion peak at m/e 221. The m/e 221 peak was the same as that observed with tirandamycin and was assigned to the dienoyl tetramic acid moiety. The spectrum of 1 showed the molecular ion peak at m/e 517 and a base peak m/e 235, each being 14 mass units

higher than those of 2, while an intense peak at m/e 283 was the same as that observed with 2. In the mass spectrum of 3, the base peak at m/e 221 was the same as that of 2, while the molecular ion at m/e 489 and an intense peak at m/e 269 were 14 mass units lower than those of 2.

Table 2 Assignment of ^{1}H-NMR spectra of Bu-2313 A and B, 4 and 8

Proton	Chemical shift δ in ppm (multiplicity and J in Hz)			
	Bu-2313 A(1)	Bu-2313 B(2)	4	8
8-CH_3	0.86(d,7)	0.87(d,7)	0.87(d,7)	0.87(d,7)
6-CH_3	1.05(d,7)	1.03(d,7)	1.03(d,7)	1.03(d,7)
15-CH_3	1.32(d,6)	1.31(d,6)	1.31(d,6)	1.32(d,6)
12-CH_3	1.42(s)	1.42(s)	1.42(s)	1.42(s)
4-CH_3	1.90(s)	1.87(s)	1.79(br.s)	1.81(s)
8-H	2.0(m)	2.07(m)	2.07(m)	2.07(m)
11-CH_2	2.44 & 2.98 (ABq,17)	2.56 & 2.95 (ABq,17.5)	2.56 & 2.95 (ABq,17.5)	2.6 & 2.9 (ABq,16)
6-H	(2.8(m)	2.76(m)	2.76(m)	2.76(m)
14-H	2.91(d,8.5)	2.92(d,7.5)	2.92(d,7.5)	2.92(d,7.5)
N-CH_3	3.03(s)	———	———	———
7-H	3.42(dd,11&2)	3.36(dd,11&2)	3.36(dd,11&2)	3.37(dd,11&2)
5'-CH_2	3.69(s)	3.76(s)	———	———
$COOCH_3$	3.78(s)	3.76(s)	3.76(s)	3.76(s)
9-H	3.99(d,5)	4.01(d,5)	4.01(d,5)	4.01(d,5)
15-H	4.48(dq,8.5&6)	4.49(dq,7.5&6)	4.49(dq,7.5&6)	4.49(dq,7.5&6)
5-H	5.97(br.d,10)	5.96(br.d,10)	5.89(d,10)	5.90(d,10)
2-H	6.98(d,15.5)	6.96(d,15.6)	5.72(d,15.5)	5.91(d,15)
NH	———	6.98(br)	———	———
3-H	7.42(d,15.5)	7.41(d,15.6)	7.25(d,15.5)	7.03(d,15)
COOH	———	———	9.85(br.s)	———
Ar	———	———		7.4 & 7.64 (ABq,8.5)

In support of the presence of the α,β;γ,δ-dienoyl chromophore the NMR of 2 (Table 2) showed two sharp doublets at low field (J=15.6 Hz) at δ 6.96 (2-H) and δ 7.41 (3-H) owing to vicinal trans protons on the α,β-unsaturated carbonyl with no proton in the γ-position. The γ-methyl (4-CH_3) signal was found at δ 1.87 and the olefinic δ-proton (5-H) appeared at δ5.96 as a doublet (J=10 Hz) broadened by coupling with the γ-methyl protons. The above-mentioned signals were also observed in the spectrum of 1. A two-proton methlene singlet (5'-CH_2) was observed in the NMR of both 1 and 2.



This methylene signal and the amide proton mentioned above were assigned to those of the tetramic acid moiety in comparison with the NMR spectrum of tirandamycin.[7]

Periodate oxidation of 2 afforded the dienoic acid 4 (mp 130-132 °C, $C_{22}H_{30}O_8$) which retained three >CH-CH$_3$ (δ 0.87, 1.03 and 1.31), two >C-CH$_3$ (δ 1.42 and 1.79), one COOCH$_3$ (δ 3.76) and three olefinic protons at δ 5.72 and 7.25 (AB quartet, J=15.5 Hz) and at 5.89 (broad doublet, J=10 Hz). The UV absorption resulting from a γ,δ-disubstituted trans,trans-α,β;γ,δ-dienoic acid occurred at 257 nm (ε 26,000), which is consistent with those of tirandamycin acid[7] and streptolic acid.[10] The carboxylic acid (4) was hydrolyzed with dil.NaOH-MeOH to give the dibasic acid 5 (mp 239-240 °C

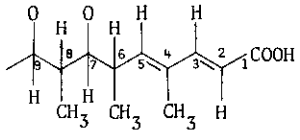
Table 3 Spin decoupling experiment on 4

Proton (δ in ppm) Irradiated		Observed		Multiplicity change	Splitting decoupled, Hz
6-H	2.76	6-CH_3	1.03	d → s	7.0
		5-H	5.89	d → s	10.0
		7-H	3.36	dd → d	2.0
8-H	2.07	7-H	3.36	dd → d	11.0
		8-CH_3	0.87	d → s	7.0
		9-H	4.01	d → s	5.0
15-CH_3	1.31	15-H	4.49	dq → d	6.0
15-H	4.49	15-CH_3	1.31	d → s	6.0
		14-H	2.92	d → s	7.5
14-H	2.92	15-H	4.49	dq → q	7.5

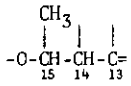
$C_{21}H_{28}O_8 \cdot 3/4H_2O$), whose IR and NMR spectra showed a lack of carbonyl at 1740 cm^{-1} and O-methyl signal at δ 3.76 owing to the $COOCH_3$ group.

The NMR spin-decoupling study on 4 (Table 3) showed that 4 had the partial structures, A and B. When a multiplet at around δ 2.76 (6-H) was irradiated, two doublets at δ 1.03 (6-CH_3) and at δ 5.89 (5-H) changed to two singlets and, in addition, a double doublet at δ 3.36 (7-H) collapsed to a doublet with J=11.0 Hz. The 7-H signal also changed to a doublet with J=2.0 Hz by irradiation at δ 2.07 (8-H). The same irradiation affected two doublets at δ 0.87 (8-CH_3) and δ 4.01 (9-H) to become singlets. The deshielded chemical shifts of 7-H and 9-H suggested that the 7- and 9-carbons should be linked to oxygen atoms.

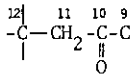
The second structural unit B was deduced also from the results of spin-decoupling experiment (Table 3). The irradiation of methyl doublet at δ 1.31 (15-CH_3) changed a low field methine signal (15-H, δ 4.49) from a double-quartet to a clear doublet. Furthermore, each of two doublets at δ 1.31 (15-CH_3) and δ 2.92 (14-H) collapsed to singlets by the irradiation at δ 4.49 (15-H), indicating that C-13 had no proton. The C-15, which bears a proton appearing at lower field (δ 4.49), should be linked with an oxygen atom other than that of an epoxide on which a methine proton



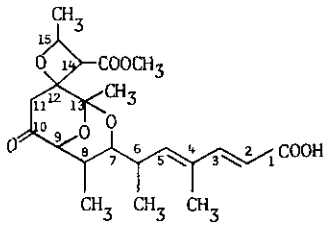
A



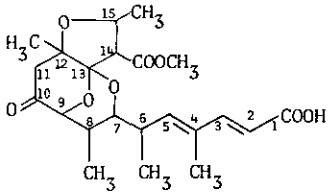
B



C



D



E (4)

usually appears at around 3 ppm. Chemical shift of 14-H (δ 2.92) implies that C-14 links with an sp^2 carbon or forms an oxirane ring with C-13.

A further three-carbon unit C adjacent to C-9 in the structure of 4 was deduced from the NMR data of the $NaBH_4$ reduction product 6 (mp 217-218 °C, $C_{22}H_{32}O_8 \cdot 1/4H_2O$) and its acetyl derivative 7 (mp 147-148 °C, $C_{24}H_{34}O_9$). The doublet at δ 4.01 owing to 9-H changed to a multiplet in 6 (δ 3.90) and 7 (δ 4.00). A new one-proton multiplet appeared in the spectrum of 6 at around δ 5.25 in the O-acetate (7). In addition, two doublets at δ 2.56 and δ 2.95 with a large J value (17.5 Hz) owing to the isolated, geminal methylene protons (11-CH_2) of 4, collapsed to multiplets and shifted to around δ 1.8 in 6 and δ 2.2 in 7. Assembling of the structural units A, B and C together with a tertiary carbon-linked methyl and a methoxycarbonyl gives the compound 4 the two possible structures, D and E.

^{13}C-NMR spectra of Bu-2313 B, tirandamycin and the dienoic acid (4) were recorded on a Varian FT-80 spectrometer at 20 MHz in $CDCl_3$. The chemical shifts of these compounds were tentatively assigned as shown in Table 4 by comparison of each spectrum side by side and in consideration of the multiplicities of signals obtained by single frequency off resonance decoupling spectra. Comparison of the spectra of Bu-2313 B and tirandamycin shows that the signals assigned to the dienoyltetramic acid moiety are in good agreement within ± 0.2 ppm deviation. This gave another proof that the structure of Bu-2313 B is the same as that of tirandamycin in the dienoyltetramic acid moiety and different in the tricyclic moiety. On the other hand, chemical shifts of Bu-2313 B were coincident with those of 4 within ± 0.1 ppm deviation in the carbons assigned to the tricyclic ring system with slightly different values for those of the dienoyl moiety. This shows that 4 is differs from Bu-2313 only in lacking the tetramic acid moiety. It retains the ring system of Bu-2313 intact with the same stereochemistry, the carbons in the dienoyl part being influenced to some extent through the conjugate system by the C-1 position.

The signal which was ascribed to the ketal carbon at the 13-position resonated at δ 96.9 in tirandamycin, whereas the signal occurred at δ 107 in Bu-2313 B and 4, about 10 ppm lower that of tirandamycin. In the structure D in which the ketal carbon (C-13) has the same type of

Table 4 ^{13}C-NMR assignment of Bu-2313 B(2), tirandamycin and related compound (4)

	Carbon	Chemical shift δ in ppm from TMS (multiplicity) Bu-2313 B(2)[c]	Tirandamycin[c]	Δδ[a]	4[c]	Δδ[b]
Dienoyl moiety	1	176.7(s)*	176.7(s)*	0	172.6	4.1
	2	116.8(d)	116.8(d)	0	115.9	0.9
	3	149.4(d)	149.5(d)	-0.1	151.4	2.0
	4	135.0(s)	134.9(s)	0.1	133.7	1.3
	5	143.3(d)	143.5(d)	-0.2	141.1	2.2
	6	34.8(d)	34.7(d)	0.1	34.5	0.3
	4-CH_3	12.3(q)**	12.3(q)**	0	12.2**	0.1
	6-CH_3	11.6(q)**	11.4(q)**	0	11.6**	0
Tricyclic ring	7	79.0(d)	78.8(d)	0.2	79.1	-0.1
	8	35.5(d)	34.5(d)	1.0	35.5	0
	9	79.0(d)	77.0(d)	2.0	78.9	0.1
	10	208.5(s)	202.4(s)		208.5	0
	11	52.1(t)	61.2(d)		52.1	0
	12	81.7(s)	57.1(s)		81.7	0
	13	107.0(s)	96.9(s)		107.0	0
	14	63.0(d)			62.9	0.1
	15	72.8(d)			72.9	-0.1
	8-CH_3	16.7(q)**	16.9(q)**	-0.2	16.7**	0
	12-CH_3	21.5(q)***	15.6(q)		21.5	0
	13-CH_3		22.6(q)			
	14-$COOCH_3$	52.3(q)			52.2	0.1
	14-$COOCH_3$	169.3(s)			169.3	0
	15-CH_3	20.0(q)***			20.0	0
Tetramic acid moiety	2'	174.9(s)*	175.0(s)*	-0.1		
	3'	100.3(s)	100.3(s)	0		
	4'	192.8(s)	192.9(s)	-0.1		
	5'	51.8(t)	51.8(t)	0		

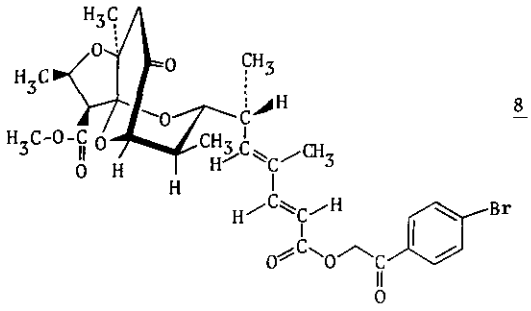
a: Difference of chemical shifts between 2 and tirandamycin.

b: Difference of chemical shifts between 2 and 4.

c: Asterisks indicate interchangeable assignments in the column where they appear.

substitution as that of tirandamycin, the signal should appear at around δ 97. This could rule out the possibility of structure D for 4.

An X-ray crystallographic study was undertaken in order to confirm the fused oxolane ring structure E for 4. The p-bromophenacyl ester of 4 (8, mp 187-188 °C, $C_{30}H_{35}BrO_9$) was prepared and crystallized from ethyl acetate-methanol to afford colorless monoclinic crystals. The structure of 8 was determined by K. Sasaki[11] (Nagoya Univ.) using a Hilger-Watts four-circle diffractometer, and the absolute configuration shown below was assigned to 8.



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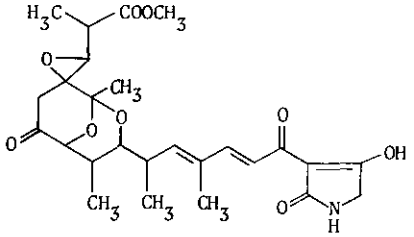
The structural integrity of compounds 4 and 8 has been evidenced by the agreement of ^{1}H- and ^{13}C-NMR spectra with those of the corresponding part of Bu-2313 (Table 2 and 4). Hence, the structure E for 4 was confirmed, which in turn established the gross structures of Bu-2313 A (1) and B (2). The structures of Bu-2313 A and B in which the dienoic acid (4) is substituted with tetramic acids have been further substantiated by the semi-synthesis[12] of Bu-2313 A and B by C-acylation of tetramic acids with 4 as described in the following chapter.

After the present work had been completed we found that a new member of this family of antibiotics named nocamycin was reported in a Russian journal.[13,14] The reported molecular formula nocamycin ($C_{26}H_{33}NO_9$) is coincident with that of Bu-2313 B and, in addition, the pysico-chemical properties and spectral data of nocamycin are very close to those of Bu-2313 B. A recent publication[15] gave nocamycin a tricyclic ketal structure including a spiro oxirane ring shown below.

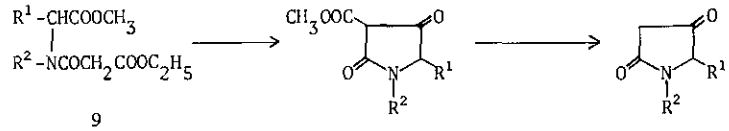


Nocamycin

II Synthesis of Bu-2313 A and B, and their analogs

In the course of the structure elucidation of Bu-2313 described above, the periodate oxidation of Bu-2313 A and B yielded the dienoic acid moiety, 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 synthesize Bu-2313 analogs having improved properties by acylation of tetramic acids or their equivalents. Recently there have been several publications[16-21] dealing with the synthesis of acyltetramic acids. The synthesis of 3-dienoyltetramic acid was first reported by Lee et al.,[19] but attempted synthesis of tirandamycin was unsuccessful by their method. Jones et al.[21] described the synthesis of acyltetramic acids including a simple dienoyl derivatives with no application of semi-synthesis of streptolydigin and tirandamycin. Here we describe the first synthesis of 3-dienoyltetramic acids possessing a naturally occurring complex ring system and with antibacterial activity similar to the natural products.

Chemistry —— Tetramic acid (11a) and the substituted tetramic acids (11b-11i) were prepared according to the procedure of Lowe et al.[22] Amino acid esters were acylated with monoethyl malonate by use of DCC giving the amides (9a-9i), which were cyclized with sodium alkoxide to give the 3-alkoxycarbonyltetramic acids (10a-10ı) as shown in Table 5. Bhat et al.[17] reported that the 5-ethylidene derivative (10d) was obtained by cyclization of ethyl N-(ethoxycarbonylacetyl)-α-aminocrotonate which was prepared from ethyl α-aminocrotonate and ethoxycarbonylacetyl chloride. In our synthesis this compound was more conveniently prepared from the threonine derivative (9d') with sodium ethoxide by simultaneous cyclization and dehydration. The 5-benzylidene derivative (10j) was prepared from 10a by condensation with benzaldehyde in the presence of hydrogen chloride.[23] Removal of the ester function from the alkoxycarbonyl derivative (10a-10j) was carried out by heating in acetonitrile to give the tetramic acids (11a-11j) and the results are given in Table 5.



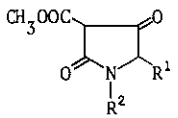
9 → 10 → 11

	R^1	R^2
a	H	H
b	H	CH_3
c	CH_3**	H
d	$CH_3CH=$	H
d'	$CH_3\underset{OH}{\underset{\vert}{CH}}$*	H
e	C_2H_5**	H
f	$CH_3S(CH_2)_2$*	H
g	$CH_3COOC(CH_2)_2$*	H
h	$CH_3(CH_2)_5$**	H
i	C_6H_5*	H
j	$C_6H_5CH=$	H

* L

** DL

Table 5 3-Carbomethoxy-5-substituted tetramic acids (10a-10j) and 5-substituted tetramic acids (11a-11j)



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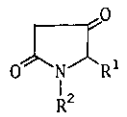

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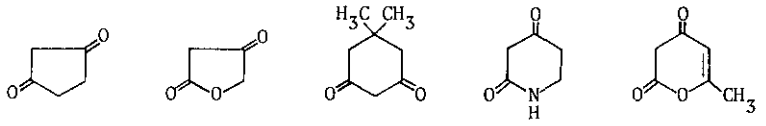
Compd.	R^1	R^2	Yield(%)	Mp (°C)	UV: $\lambda_{max}^{H_2O}$ nm(ε)	Yield(%)	Mp (°C)	UV: $\lambda_{max}^{H_2O}$ nm(ε) [h]
a	H	H	64	>300 [b]	227(13,400) 261(11,300)	100	>300 [l]	259(11,200)
b	H	CH_3	78	186-188 [e,f,g]	227(11,400) [a] sh260(3,900)	100	j,m	———
c	CH_3	H	65	152-153 [d]	228(13,500) 262.5(10,900)	100	108-114 [m]	261(11,000)
d	CH_3CH=	H	50	181-183 [e,f,g]	277(14,200) [a]	29	178-179 [f,n]	263.5(19,100) 321.5(4,200)
e	C_2H_5	H	61	182-183 [f]	229(14,000) 263(11,300)	100	106-107 [f]	262(11,900)
f	$CH_3S(CH_2)_2$	H	90	157-158 [f]	228(15,500) 263(11,800)	99	76-77 [f]	262.5(10,900)
g	$CH_3OOC(CH_2)_2$	H	59	135-136 [f]	229(14,500) 263(11,500)	100	k	———
h	$CH_3(CH_2)_5$	H	82	153.5-154.5 [f]	230(11,800) [a] 257(7,000)	100	92-93 [f]	262.5(11,700) [i]
i	C_6H_5	H	93	149.5-150 [f]	230(13,200) [a] 261(8,500)	91	128-129 [f]	267(22,300) [i]
j	C_6H_5CH=	H	19	182.5-183 [f]	232.5(13,300) [a] 327.5(24,400)	100	184-186 [f]	219(25,900) 312.5(32,300)

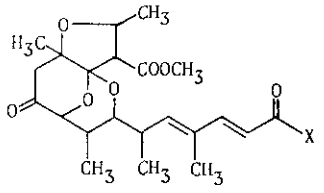
a: Run in MeOH. *b*: See ref.22. *c*. See ref.21. *d*: See ref.24. *e*: See ref.17. *f*: Elemental analyses were coincident with the calculated value within ± 0.4 % deviation. *g*: Ethyl ester. *h*: Measured in water containing a drop of 0.1 N NaOH. *i*: Measured in EtOH containing a drop of 0.1 N NaOH. *j*: Ref.23, isolated as semi-solid (lit., mp 48-51 °C), which showed the expected IR spectrum. *k*: An oil, which showed a compatible IR spectrum. *l*: See ref.23. *m*: See ref.24. *n*: See ref.17, wherein no physico-chemical data were described.

The following cyclic 1,3-dicarbonyl compounds were employed as substrates for acylation: 1,3-cyclopentanedione (12a); tetronic acid (12b)[25] which occurs as a constituent of some fungal metabolites[26,27]; dimedone (12c); 2,4-diketopiperidine (12d), the 5,6-dehydro derivative of which occurs in mocimycin,[28] efrotomycin[29] and aurodox (X-5108)[30]; 4-hydroxy-5-methyl-2-pyrone (12e), the 5,6-dihydro derivative of which is a constitutent of an antifungal methabolite, alternaric acid.[31] These compounds are commercially available except for 2,4-diketopiperidine (12d), which was prepared from β-alanine ester *via* a malonamide and a cyclic ester in a manner similar to the preparation of tetramic acids.



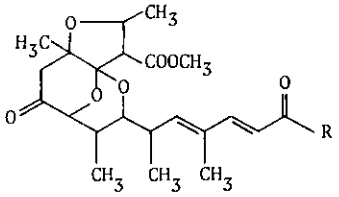
12a 12b 12c 12d 12e

The dienoic acid (4)[5] prepared by periodate oxidation of Bu-2313 A (1) and Bu-2313 B (2) was transformed into the acid chloride (4a) by the action of thionyl chloride, which was converted into the anilide (4b) in 38 % yield (colorless needles) to confirm formation of the acid chloride. Mp 117-119 °C. λ_{max}^{EtOH} 278 nm (ε 27,600). Anal. $C_{28}H_{35}NO_7 \cdot 1/4H_2O$.

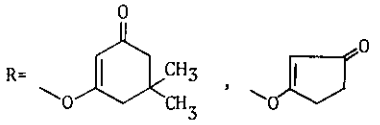


4 X = OH
4a X = Cl
4b X = NHC_6H_5

Borontrifluoride etherate,[17] triethylamine,[18] and thallium salt[19] have been used in acylation of tetramic acids. In our attempts to acylate tetramic acid (11a) with the acid chloride (4a), the use borontrifluoride etherate was found to be unsuitable because of extensive degradation of 4a during the reaction. Acylation with 4a using triethylamine did not give the desired product. Reaction of the acid chloride (4a) with dimedone, 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 (13a) in 87 % yield. Mp 150-151 °C. Anal. $C_{30}H_{40}O_9 \cdot 1/4H_2O$. 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 maximum of compound 13a which occurred at 276.5 nm (ε 34,100) did not show the reported pH-depending-shifts[8,9,19] characteristic of the 2-acyl-1,3-dicarbonyl systems. Acylation of 1,3-cyclopentanedione in the presence of triethylamine afforded also the



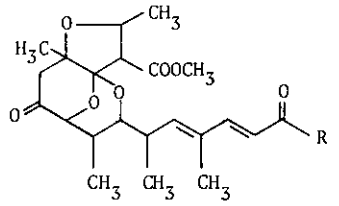
O-acylated derivatives

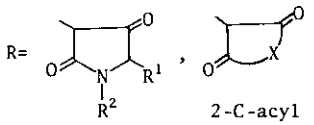


13a 13b

O-acylated product 13b in 51 % yield, mp 187-189 °C. UV: λ_{max}^{EtOH} 288 nm (ε 31,000). Anal. $C_{27}H_{34}O_9 \cdot 1/2H_2O$.

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 11a with 4a 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 acylation of N-methyltetramic acid (11b) with 4a in DMF in the presence of sodium hydride (Method B).





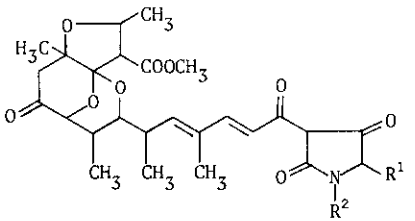
3-acyltetramic acids (14a-14j)

2-C-acyl cyclic 1,3-diketones (15a-15e)

Most of the C-acylated products in the present study (14d-14j, 15a and 15b) were prepared by Method B. Lithium hydride was effective in acylation of dimedone (12c) and 2,4-diketopiperidine (12d) with 4a in dry DMF (Method C). Acylation of 4-hydroxy-6-methyl-2-pyrone (12e) was accomplished by heating a mixture of the carboxylic acid 4 and phosphorus pentoxide in benzene (Method D) to give the desired product 15e in 5 % yield, whereas Method B gave only a trace of 15e. Tables 6 and 7 show the results of C-acylation of the tetramic acids (11a-11j) and the cyclic 1,3-dicarbonyls (12a-12e), respectively.

As indicated in tables 6 and 7, the C-acylated products, 14a-14j and 15a-15e, were characterized by a bathochromic shift of the maximum in the UV spectrum at the shortest wavelength (near 240 nm) and hypsochromic shift of the maximum at the longest wavelength (near 350 nm) on change from acidic

Table 6 Bu-2313 A, Bu-2313 B and 3-Acyl-5-substituted tetramic acids



Compd.	R^1	R^2	Method	Yield(%)	Mp (°C)	λ_{max} nm (ε) Acidic[a]	λ_{max} nm (ε) Basic[b]	^{1}H-NMR signals of tetramic acid moiety δ in ppm
14a	H (Synthetic Bu-2313B)	H	A	11	130-133[c]	237(10,500) 354(34,300)	255(15,700) 285(18,900) 332(19,900)	3.69 (s, 5'-CH_2)
14b	H (Synthetic Bu-2313A)	CH_3	B	6	103-105[g]	242.5(8,900) 357.5(30,900)	264(17,900) 288(17,800) 336(17,400)	3.01 (s, N-CH_3) 3.68 (s, 5'-CH_2)
14c	CH_3	H	B	50	133-135[g]	236(7,600) 352(33,500)	255(14,200) 283(16,900) 330(18,700)	1.37(d,J=7Hz, 5'-CH_3)
14d	CH_3CH=	H	B	2	120-123[d]	247(14,100) 280(18,600) 356(40,100)	258(21,400) 294(33,000) 346(17,700)	1.79 (m, =CH-CH_3) 5.25(q,J=6.5Hz,=CH-CH_3)
14e	C_2H_5	H	B	34	109-113[e]	238(9,600) 354(34,000)	257(15,000) 285(7,600) 334(19,200)	0.99(t,J=7Hz, CH_2CH_3)

Table 6 Bu-2313 A, Bu-2313 B and 3-Acyl-5-substituted tetramic acids (cont'd)

Compd.	R^1	R^2	Method	Yield(%)	Mp (°C)	λ_{max} nm (ε) Acidic[a]	λ_{max} nm (ε) Basic[b]	^{1}H-NMR signals of tetramic acid moiety δ in ppm
14f	$CH_3S(CH_2)_2$	H	B	13	137-140[g]	238(8,000) 353(29,400)	258(15,300) 279(17,500) 331(16,000)	2.01 (s, S-CH_3)
14g	$CH_3OOC(CH_2)_2$	H	B	30	132-135[g]	237(7,600) 353(25,900)	260(13,900) 279(17,200) 330(14,000)	3.65 (s, $COOCH_3$)
14h	$CH_3(CH_2)_5$	H	B	6	103-109[g]	236(8,100) 353(31,200)	256(16,000) 280(18,700) 331(17,500)	0.90 (m, $C_5H_{10}\underline{CH_3}$)
14i	C_6H_5	H	B	8	126-129[g]	240(8,200) 355(28,400)	260(14,800) 281(15,500) 333(17,000)	7.25 (s, C_6H_5)
14j	$C_6H_5CH=$	H	B	6	148-153[f]	227(11,100) 360(44,900)	243(14,000) 293(26,800) 333(40,600)	7.29 (s, C_6H_5)

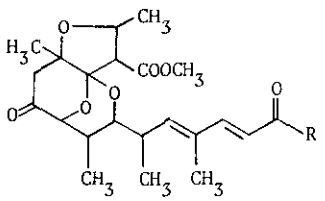
a: 0.001 N HCl in 99 % ethanol. b: 0.001 N NaOH in 99 % ethanol.

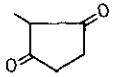
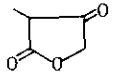
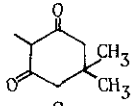
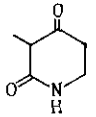
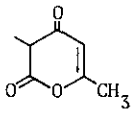
c: Molecular weight was confirmed by mass spectrometry; M^+ 503.

d: M^+ 529. e: $M^+ + 1$ 532. f: M^+ 591.

g: Microanalyses were coincident with the calculated value within ± 0.4 % deviation.

Table 7 2-C-Acylated cyclic 1,3-dicarbonyl derivatives



Compd.	R	Method	Yield(%)	Mp (°C)	λ_{max} nm (ε) Acidic[a]	λ_{max} nm (ε) Basic[b]	^{1}H-NMR signals of 1,3-dicarbonyl moiety δ in ppm
15a		B	4	103-106[d]	228(11,900) 357(24,000)	248(22,000) 292(17,400) 322(17,900)	2.6 (m, CO-CH_2CH_2-CO)
15b		B	16	93-97[e,g]	232(6,000) 350(22,600)	239(14,800) 313(16,800)	4.40 & 4.65 (ABq, J=12Hz, O-CH_2-CO)
15c		C	20	68-70[g]	251(13,000) 346(21,200)	271(31,300) 328(7,900)	1.13(s, $C<^{CH_3}_{CH_3}$) 17.0 (s,OH)
15d		C	13	103-106[g]	246(9,900) 346(29,400)	264(23,700) 333(14,900)	3.45(t,J=7Hz,NH-CH_2CH_2-CO) 2.63(t,J=7Hz,NH-CH_2CH_2-CO)
15e		D	5	98-108[f,g]	241(10,500) 353(24,600)	270(17,300) 330(11,200)	2.24 (s, CH_3) 5.84 (s, >C=CH)

a: 0.001 N HCl in 99 % ethanol. b: 0.001 N NaOH in 99 % ethanol. c: DMSO was used in addition to DMF.

d: Molecular weight was confirmed by mass spectrometry; M^+ 502. e: $M^+ + 1$ 505. f: M^+ 530.

g: Microanalyses were coincident with the calculated value within ± 0.4 % deviation.

to basic solution.[8,9,19] Magnitude of the shifts varies with the β-tricarbonyl moieties involved. The bathochromic shift of 18-23 nm and the hypsochromic shift of 20-23 nm were observed in the 3-acyltetramic acids except 14d and 14j, 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 similar to the original antibiotics,[5] together with distinguishable peaks owing to the β-tricarbonyl moieties involved.

Antibacterial Activity

Table 8 shows the *in vitro* activity of synthetic Bu-2313 A and B, and their analogs against two anaerobes (*B. fragilis* A20926 and *P. acne* A21933) and an aerobe (*S. Pyogenes* A9604). The data presented are representative of those obtained from a collection of 12 anaerobic and 32 aerobic test organisms 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 Bu-2313. The synthetic Bu-2313 A and B are as active as the corresponding natural antibiotics. In the 3-acyltetramic acids (14a-14j), an increase in the chain length of the 5'-substitutent led to a diminution in activity, especially against *S. pyogenes*. The 5'-methyl derivative (14c) is the most active among the semi-synthetic analogs against the 3 strains cited in Table 13 and more active than Bu-2313 A against *B. fragilis* and *S. pyogenes*, although it is a half as active as Bu-2313 B. The 5'-ethyl derivative (14e) showed activity comparable to Bu-2313 A.

A similar antibacterial spectrum was shown by 2-acyl-1,3-dicarbonyl derivatives (15a-15e) possessing ring systems different from the naturally occurring tetramic acid. The 1,3-cyclopentanedione derivative (15a), the most active member of this series, showed better antibacteroides activity than Bu-2313 A with the same level of activity against *P. acne* and *S. pyogenes*.

Table 8 *In vitro* activity of synthetic Bu-2313 and analogs

	MIC (mcg/ml)		
	B.fragilis A20926	*P.acne* A21933	*S.pyogenes* A9604
14a synthetic Bu-2313B	0.05	0.2	0.8
14b synthetic Bu-2313A	0.4	0.4	3.1
14c	0.1	0.4	1.6
14d	6.3	0.8	12.5
14e	0.1	0.8	3.1
14f	0.2	0.8	6.3
14g	0.4	1.6	12.5
14h	1.6	3.1	100
14i	1.6	1.6	1.6
14j	0.1	1.6	25
15a	0.1	0.4	6.3
15b	25	6.3	12.5
15c	6.3	1.6	25
15d	3.1	0.8	3.1
15e	12.5	3.1	25
Bu-2313 A	0.2	0.2	6.3
Bu-2313 B	0.05	0.2	0.8

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