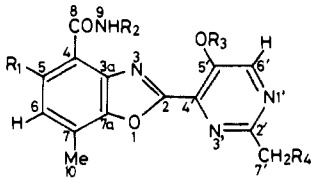
Structure of the Novel Antibiotics Boxazomycins A, B, and C

Takenori Kusumi,[†] Takashi Ooi,[†] Markus R. Wälchli,[‡] and Hiroshi Kakisawa*[†]

Contribution from the Department of Chemistry, The University of Tsukuba, Sakura, Ibaraki, Japan 305. *Received July 7, 1987*

Abstract: Structures of three new antibiotics, boxazomycins A, B, and C, were determined on the basis of their spectral properties, including modern NMR techniques such as INADEQUATE and NH-COLOC spectra. Observation of long-range couplings between phosphorus and carbon atoms of a phosphate derivative of boxazomycin A was helpful for the structure elucidation. Boxazomycins have a novel 7-methyl-2-(2-methylpyrimidin-4-yl)benzoxazole-4-carboxylic acid skeleton.

Boxazomycins A (**1**) and B (**2**) are antibiotics produced by Actinomycetes strain No. G495-11, which was isolated from a soil sample in Taiwan.[1] They are highly active in vitro against



1 $R_1 = R_4 = OH$, $R_2 = R_3 = H$
2 $R_1 = OH$, $R_2 = R_3 = R_4 = H$
3 $R_1 = R_2 = R_3 = H$, $R_4 = OH$
4 $R_1 = R_4 = OAc$, $R_2 = R_3 = Ac$
5 $R_1 = OAc$, $R_2 = R_3 = Ac$, $R_4 = H$
6 $R_1 = R_2 = H$, $R_3 = Ac$, $R_4 = OAc$
7 $R_1 = OH$, $R_2 = H$, $R_3 = PO(OEt)_2$, $R_4 = OPO(OEt)_2$
8 $R_1 = R_4 = OAc$, $R_2 = Ac$, $R_3 = Me$
9 $R_1 = R_4 = OAc$, $R_2 = CHO$, $R_3 = Me$
10 $R_1 = H$, $R_2 = CHO$, $R_3 = Me$, $R_4 = OAc$

gram-positive bacteria and anaerobes such as *Staphylococcus aureus*, *Streptococcus pyogenes*, *Mycobacterium smegmatis*, *Clostridium difficile*, and *Bacterioides fragilis*.[1] Our study on the structure elucidation of boxazomycins has long been fruitless because (1) ordinary ^1H and ^{13}C NMR spectral analyses were not helpful owing to the too simple features of the spectra, (2) they gave no identifiable products by degradation reactions under various conditions, and (3) they and their derivatives did not afford good crystals for X-ray analysis. Recently, we were able to elucidate their structures by means of modern NMR techniques, and this report describes the structures of boxazomycins A and B together with that of boxazomycin C (**3**), a newly isolated congener.

Boxazomycin A (**1**), $C_{14}H_{12}N_4O_5$, is insoluble in ordinary organic solvents and, therefore, converted into tetraacetate **4**. The ^1H NMR spectrum of **4** gave only nine singlets, as shown in Figure 1. Of the five methyl singlets, the most deshielded one at δ 2.68 is rather broad and, by a double-resonance experiment, this methyl signal was found to be coupled with the aromatic proton at δ 7.07. The other four singlets are ascribable to acetyl groups. Also, there is a sharp methylene singlet at δ 5.37, possibly assignable as the methylene protons of the CH_2OAc moiety, and a highly deshielded aromatic proton at δ 8.67. The signal at δ 10.7 is broad and exchangeable with D_2O and was deduced to be a hydroxyl or amide proton signal.

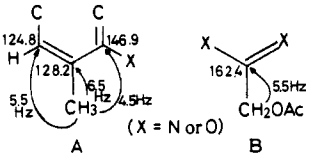
With the aid of LSPD (long-range selective proton decoupling),[2] C–H COSY, and 2D-COLOC[3,4] experiments, all the proton signals could be correlated with the ^{13}C signals, and the coupling constants were firmly established (Tables I and II). The methyl protons appearing at δ 2.68 are coupled with a tertiary carbon (δ 124.8) and two quaternary carbons (δ 128.2 and 146.9). The downfield chemical shift (δ 146.9) of one of the quaternary carbons

Table I. ^{13}C and ^1H Chemical Shifts for **4** (δ from TMS)

	carbon		proton	
	$CDCl_3$	DMSO-d_6	$CDCl_3$	DMSO-d_6
2	158.1	157.9		
3a	139.2	138.9		
4	113.1	117.8[a]		
5	149.5	145.9		
6	124.8	123.9	7.07	7.48
7	128.2	125.5		
7a	146.9	146.7		
8	160.5[a]	162.4[a]		
9			10.7[b]	11.3[b]
10	15.4	15.1	2.68	2.65
2′	162.4	162.6		
4′	143.5	143.9		
5′	142.7	142.5		
6′	154.7	155.4	8.67	9.11
7′	65.1	65.2	5.37	5.42
5-OAc	169.6	168.9[c]		
	20.5	20.6	2.30	2.33[d]
9-Ac	172.7[a]	171.0[a]		
	25.2	25.0	2.50	2.45
5′-OAc	168.4	169.0[c]		
	20.6	20.6	2.38	2.33[d]
7′-OAc	170.4	170.3		
	20.5	20.6	2.17	2.25[d]

[a] Isotope shift doubling is observed in the presence of CD_3OD. [b] CD_3OD exchangeable. [c,d] Assignments may be interchanged.

suggests that this carbon possesses a heteroatom (N or O). These findings led to the partial structure A. In contrast, the acetoxymethyl protons (δ 5.37) are long-range coupled with only one quaternary carbon (δ 162.4), suggesting that both sides of the quaternary carbon are blocked by heteroatoms (N or O) (partial structure B). However, no further information about the structure was obtained from these experiments, because it was impossible to deduce whether the long-range coupling constants of the other protons (δ 7.07, 8.67, and 10.7) corresponded to $^2J_{CH}$, $^3J_{CH}$, or $^4J_{CH}$ in such a highly oxidized aromatic compound. Since the homonuclear (^1H–^1H) and heteronuclear (^1H–^{13}C) couplings were not so informative at this stage, we turned our attention to a ^{13}C–^{13}C double quantum coherence spectrum. Delightfully, the



[†] The University of Tsukuba.
[‡] Bruker Japan Co. Ltd.

(1) Saito, K.; Nakakita, Y.; Hatori, M.; Tomatsu, K.; Tomita, K.; Miyaki, T.; Konishi, M.; Kawaguchi, H., *J. Antibiot.*, in preparation.
(2) Seto, H.; Sakai, T.; Yonehara, H.; Uzawa, J. *Tetrahedron Lett.* **1978**, 923.
(3) Kessler, H.; Bermel, W.; Griesinger, C. *J. Am. Chem. Soc.* **1985**, *107*, 1083.
(4) Kessler, H.; Griesinger, C.; Zarbock, J.; Loosli, H. R. *J. Magn. Reson.* **1984**, *57*, 331.

0002-7863/88/1510-2954$01.50/0

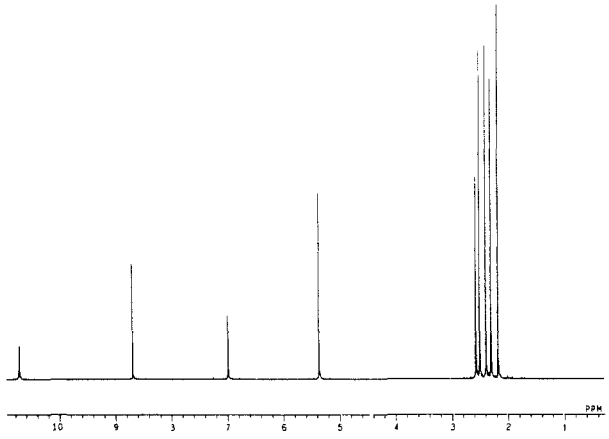


Figure 1. 400-MHz ^1H NMR spectrum of **4** in $CDCl_3$ at 298 K.

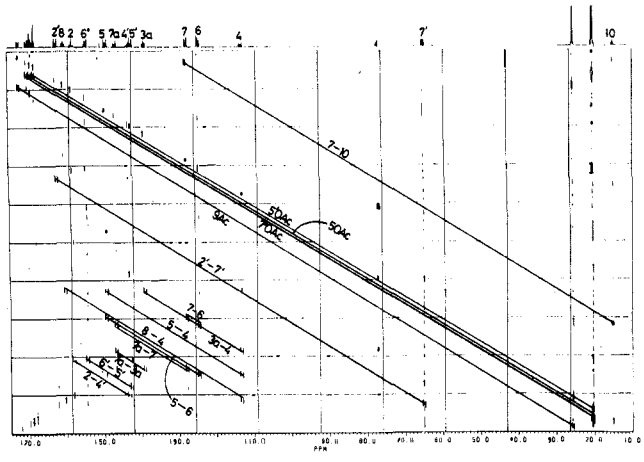
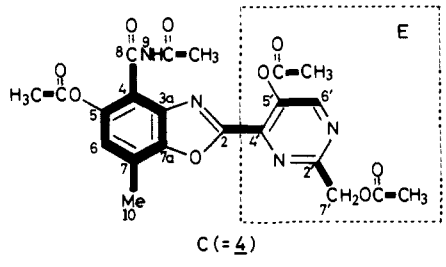


Figure 2. 2D-INADEQUATE spectrum of **4** (600 mg) in $CDCl_3$ in a 10-mm tube at 318 K. Correlation peaks are due to direct-bond connectivity between ^{13}C nuclei. Lines have been added for clarification of the signal assignment.

2D-INADEQUATE spectrum[5-7] of the tetraacetate **4** (Figure 2) exhibited almost all the double quantum coherent peaks, from which the C–C connectivities depicted by thick lines in structure C were derived without difficulty. For instance, partial structure



C (= 4)

A was clearly supported by the correlation peaks δ 15.4 (C-10)–δ 128.2 (C-7), δ 128.2 (C-7)–δ 124.8 (C-6), and δ 128.2 (C-7)–δ 146.9 (C-7a). Moreover, this structure was expanded to the methylbenzoyl group (left half of C) by tracing the coherent peaks δ 15.4 (C-10)–δ 128.2 (C-7)–δ 124.8 (C-6)–δ 149.5 (C-5)–δ113.1 (C-4)–δ 139.2 (C-3a)–δ 146.9 (C-7a)–δ 128.2 (C-7) and δ 113.1 (C-4)–δ 160.5 (C-8). However, there are still some isolated fragments (right half of C). Connectivity between C-4′ and -5′ (The C-4′/C-5′ connectivity peaks are not observed in the 2D-INADEQUATE spectrum because of their similar chemical shifts, δ 143.5 and 142.7, respectively.) was determined by the following experiments. Boxazomycin A (**1**) was converted into the diphosphate **7** by treatment with diethyl chlorophosphate in the presence of 2,6-dimethylpyridine. The ^{13}C NMR spectrum of **7**

(5) Bax, A.; Freeman, R.; Frenkiel, T. A. *J. Am. Chem. Soc.* **1981**, *103*, 2102.
(6) Freeman, R.; Frenkiel, T. A. *J. Am. Chem. Soc.* **1982**, *104*, 5545.
(7) Mareci, T. A.; Freeman, R. *J. Magn. Reson.* **1982**, *48*, 158.

Table II. Selected ^1H–^{13}C Coupling Constants (Hertz) for **4** in $CDCl_3$

from proton	to carbon	J,[a] Hz
	^1J	
6	6	162.0
10	10	129.5
6′	6′	187.5
7′	7′	143.5
5-OAc (Me)	5-OAc (Me)	130.5
9-Ac (Me)	9-Ac (Me)	130.5
5′-OAc (Me)	5′-OAc (Me)	130.5
7′-OAc (Me)	7′-OAc (Me)	130.5
	$\geq^2J$	
6	3a	*
	4	5.5
	5	3.5
	7a	12.0
	8	*
	10	4.5
9	4	*
	5	*
	8	2.0
	9-Ac (C=O)	1.5
	(Me)	3.0
10	3a	*
	4	*
	6	5.5
	7	6.5
	7a	4.5
6′	2	2.0
	2′	11.0
	4′	4.0
	5′	2.5
	5′-OAc (C=O)	*
7′	2′	5.5
	4′	*
	5′	*
	6′	*
	7′-OAc (C=O)	4.5
	(Me)	*
5-OAc (Me)	5	*
	5′OAc (C=O)	7.0
9-Ac (Me)	9-Ac (C=O)	7.0
5′-OAc (Me)	5′	*
	5′-OAc (C=O)	7.0
7′-OAc (Me)	7′	*
	7′-OAc (C=O)	7.0

[a] Small (1-Hz) couplings (*) were detected in the CH-COLOC experiments.

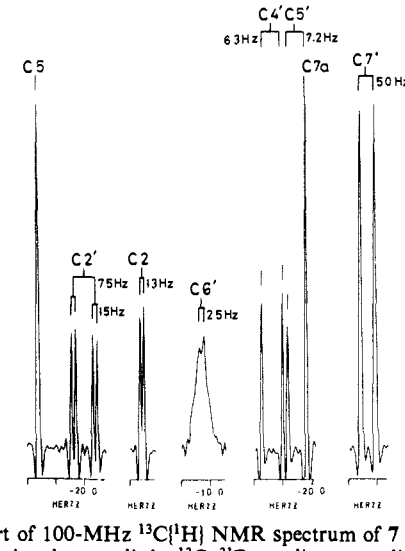
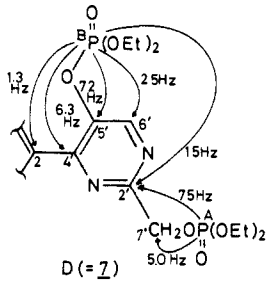


Figure 3. Part of 100-MHz $^{13}C\{^1H\}$ NMR spectrum of **7** in $CDCl_3$ at 298 K. Some signals are split by ^{13}C–^{31}P couplings; coupling constants (J_{CP}) are presented.

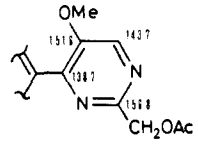
revealed that the chemical shifts of the carbon signals are similar to those of **4**, and the assignments of the carbon signals could be done by LSPD experiments. Introduction of one phosphate group (P_A) at the hydroxymethyl group (7′-CH_2OH) was deduced from the coupling of the methylene carbon signal (C-7′) and the quaternary carbon signal (C-2′) from a phosphorus atom (P_A), and the location of the other phosphate group (P_B) at C-5′–O was inferred by the splitting of the C-5′ owing to the coupling from another phosphorus atom (P_B) (see D and Figure 3). The rel-



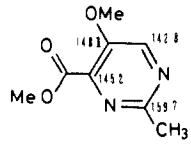
D (= 7)

atively large coupling constants between C-4′ and P_B ($^3J_{CP}$ = 6.3 Hz) shows that C-4′ is directly bonded to a carbon possessing an O–P group (C-5′). Surprisingly, carbon 2′ was found to be coupled with both the phosphorus atoms (P_A and P_B). The coupling from P_B to C-2′ (^3J)[8] implied that C-2′ was located in the vicinity of C-5′. On the basis of these findings and partial structure B, the expanded partial structure E (right half of C) was proposed. It should be noted that the coupling constant between P_B and C-4′ (6.3 Hz) is larger than that between P_B and C-6′ (2.5 Hz). The significant difference of the coupling constants indicates that the phosphate moiety at C-5′ takes the conformation in which the phosphorus atom (P_B) is located trans to C-4′ (large transoid three-bond coupling, ^3J) and cis to C-6′ (small cisoid ^3J) with regard to the C-5′–O bond, as illustrated in partial structure D.[8]

The presence of the pyrimidine moiety E in the tetraacetate **4** was supported by the ^{15}N NMR spectrum; the proton noise decoupled spectrum exhibited four singlets (Figure 4a). The chemical shifts of the two downfield signals (δ 303.5 and 287.6) are compatible with those reported for the nitrogen atoms of pyrimidine derivatives.[9-12] In the proton-coupled spectrum (Figure 4b), the signal at δ 287.6 (N-3′) appears as a finely split triplet (J = 1.5 Hz), indicating the coupling from the methylene protons at C-7′. Another signal [δ 303.5 (N-1′)] is split into a double triplet (J = 10.5, 1.5 Hz) owing to the coupling with both 6′-H and 7′-H_2. Also, the pyrimidine moiety E was reinforced by comparison of the ^{13}C NMR spectrum of the compound **8**, which



8



11

was obtained by reaction of boxazomycin A (**1**) with diazomethane followed by acetylation, with that of the synthetic compound **11**. The chemical shifts of the aromatic carbons of **8** are very close to those of the corresponding carbons in **11**, as shown in the respective structures.

The largest fragment that could be confirmed by the 2D-INADEQUATE spectrum was the 4-methylbenzoyl group. From the chemical shifts, it is obvious that the carbons at 3a (δ 139.2),

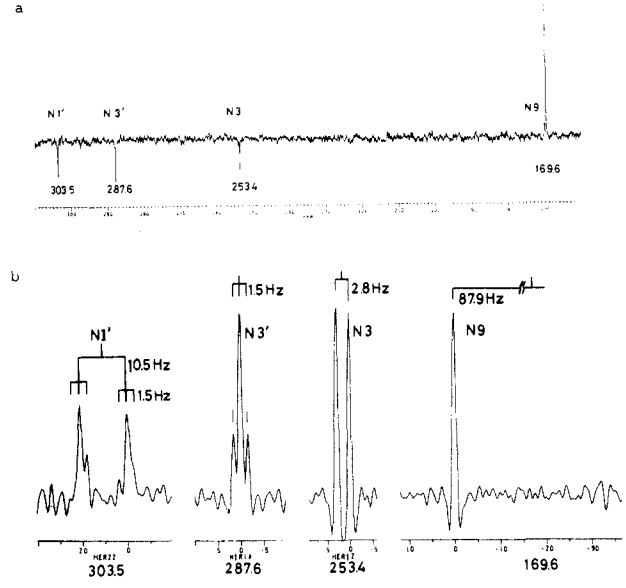
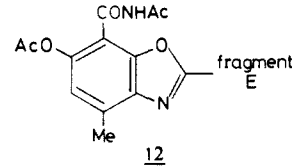


Figure 4. 40-MHz ^{15}N NMR spectrum of **4** (600 mg) in $CDCl_3$ at 318 K. Chemical shifts (δ) are referred to external CH_3NO_2 (δ 379.6). (a) Proton noise decoupling spectrum. The phase of the chart is, for convenience, inverted to 180°. Thus, the N_9 signal has a negative intensity due to negative NOE in the real spectrum. (b) Proton-coupled spectrum. Coupling constants (J_{NH}) are presented.

7a (δ 146.9), and 5 (δ 149.5) (see C) must be connected with oxygen or nitrogen atoms. Of the three, C-5 is correlated with acetyl methyl protons (δ 2.30) by a CH-COLOC experiment, and thus C-5 must possess an acetoxy group. When the ^{13}C NMR spectrum of **4** was measured in DMSO-d_6 in the presence of an equimolar amount of CD_3OD, the signals of C-8 (δ 162.4) and C-4 (δ 117.8) as well as an acetyl carbon (δ 171.0) changed into two peaks due to partial exchange of NH to ND,[13] suggesting an occurrence of an imide group at C-4. The remaining one nitrogen and one oxygen atoms, which are not assigned yet, of the tetraacetate **4** have to be bonded to C-3a and C-7a, respectively, or the reverse. The above facts allowed the assignment of the structure **4** or **12** for the tetraacetate of boxazomycin A.



12

In the proton noise decoupled ^{15}N spectrum (Figure 4a), the chemical shifts of the two signals at δ 253.4 and 169.6 are easily interpretable as those of benzoxazole and imide nitrogens, respectively.[10-12,14] In the proton-coupled spectrum, the oxazole nitrogen signal [δ 253.4 (N-3)] is split into a doublet (J = 2.8 Hz; Figure 4b), suggesting that N-3 is coupled with either the aromatic proton H-6 or the amide proton H-9. The NH-COLOC spectrum[4] (Figure 5) clearly shows that H-9 is coupled with N-3, possibly through hydrogen bonding. This finding eliminates the possibility of structure **12**. Further evidence for structure **4** was obtained by the following experiment. In the solid-state ^{13}C NMR spectrum (CPMAS)[15-19] of the tetraacetate **4** (Figure 6), the signal of C-3a is finely split, suggesting that C-3a is connected with a nitrogen atom.[20] The other signals of the carbons bonded to

(8) Buchanam, G. W.; Wightmann, R. H.; Malaiyandi, M. *Org. Magn. Reson.* **1982**, *19*, 98.
(9) Stefaniak, L.; Roberts, J.; Witanowski, M.; Webb, G. A. *Org. Magn. Reson.* **1984**, *22*, 201.
(10) Witanowski, M.; Stefaniak, L.; Webb, G. A. *Annu. Rep. NMR Spectrosc.* **1981**, *11B*.
(11) Stefaniak, L.; Webb, G. A.; Witanowski, M. *Annu. Rep. NMR Spectrosc.* **1986**, *18*.
(12) Levy, G. C.; Lichter, R. L. *Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy*; Wiley: New York, 1979.
(13) Hansen, P. E. *Annu. Rep. NMR Spectrosc.* **1983**, *15*, 105.
(14) Stefaniak, L.; Roberts, J.; Witanowski, M.; Webb, G. A. *Org. Magn. Reson.* **1984**, *22*, 215.
(15) Pines, A.; Gibby, M. G.; Waugh, J. S. *J. Chem. Phys.* **1973**, *59*, 569.
(16) Andrew, E. R. *Int. Rev. Phys. Chem.* **1981**, *1*, 195.
(17) Schaefer, J.; Stejskal, E. O. *J. Am. Chem. Soc.* **1976**, *98*, 1031.
(18) Demco, D. E.; Tegenfeldt, J.; Waugh, J. S. *Phys. Rev. B: Solid State* **1975**, *11*, 4133.
(19) Yannoni, C. S. *Acc. Chem. Res.* **1982**, *15*, 201.

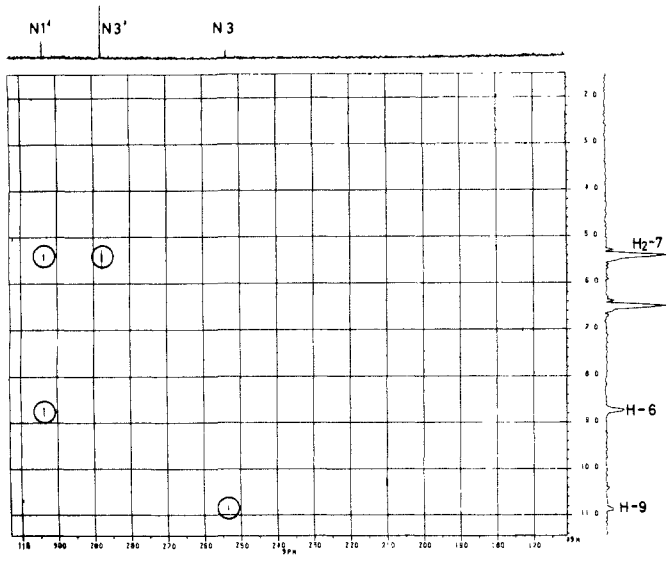


Figure 5. NH-COLOC (J_{NH} = 2 Hz) spectrum of **4** (600 mg) in $CDCl_3$ at 318 K. Circles have been added for clarification of the correlation peaks.

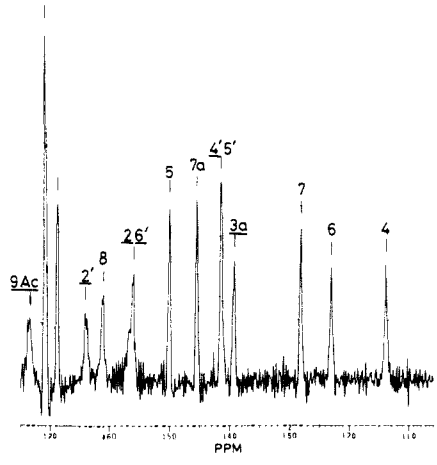


Figure 6. Downfield part of solid-state ^{13}C NMR spectrum (100 MHz) of **4**, which was obtained by using a cross-polarization and magic angle spinning (CPMAS) technique together with proton high-power decoupling. Assignment was carried out in comparison with the spectrum in solution. Carbons directly connected with nitrogens are underlined.

nitrogen atoms (9-Ac and C-2', -8, -2, and -6') are also split, which is consistent with the proposed structure **4** and the ^{13}C assignment. On the basis of these findings, we concluded that boxazomycin A has structure **1**.

Boxazomycin B (**2**) has a molecular formula, $C_{14}H_{12}N_4O_4$, that is lacking one oxygen atom in comparison with that of boxazomycin A (**1**). The ^1H NMR spectrum of **2** was almost superimposable with that of boxazomycin A (**1**), except that a sharp singlet due to a methyl group appears at δ 2.20 in the former in place of the hydroxymethyl (C-7') signal in the latter. Also, the ^{13}C NMR spectrum of the acetate (**5**) of **2** is lacking the signal due to an acetoxymethyl group and, instead, exhibits a quartet at δ 26.3 (C-7') in addition to another methyl signal at δ 15.8 (C-10). These properties, together with the UV spectrum of **2**, which is almost identical with that of **1**, led to the structure **2** of boxazomycin B.

Boxazomycin C (**3**) was isolated as a diacetate (**6**) from the crude sample of the tetraacetate **4** of boxazomycin A. The spectroscopic properties of **6** are similar to those of **4** as a whole. However, there were some significant differences in the IR and ^1H NMR spectra between the two compounds; the IR spectrum of **6** shows bands at 3390, 3180, and 1625 cm^{-1} assignable as a primary amide. Also, the ^1H NMR spectrum of **6** exhibits two doublets at δ 7.58 (J = 8 Hz, H-5) and 7.98 (J = 8 Hz, H-6) together with a singlet at δ 9.13 (H-6') in the aromatic region. These facts inferred the structure **3** for boxazomycin C. The convincing evidence for structure **3** was obtained by a degradation reaction. Acid hydrolysis (6 M HCl, 100 °C, 24 h) of **3** gave rise to 2-amino-3-hydroxy-4-methylbenzoic acid, which was identified by comparison of its physical properties with those of an authentic sample.[21]

It is noteworthy that, although the amide groups of boxazomycins A (**1**) and B (**2**) were easily acetylated (Ac_2O/pyridine, room temperature) to give the imides **4** and **5**, respectively, the amide group of boxazomycin C (**3**) resisted acetylation. This observation is in good agreement with the fact that 2-hydroxybenzamide is easily acetylated because of facile intramolecular migration of the acetyl group from the neighboring oxygen atom to the nitrogen, although benzamide is not acetylated under the same reaction conditions.

As far as we know, boxazomycins are the first natural products that have a pyrimidinylbenzoxazole skeleton.

Experimental Section

Infrared spectra were recorded on a Hitachi grating 215 infrared spectrophotometer. ^1H and ^{13}C NMR spectra were measured on JEOL FX-90Q and Bruker AM-400 and AM-500 spectrometers. ^{15}N spectra and CPMAS were recorded with a Bruker AM-400 spectrometer. Mass spectra were taken on a Hitachi RMU-6M instrument. UV spectra were measured on a Hitachi 340 spectrophotometer.

Isolation of Boxazomycins A and B.[1] The fermentation of the strain No. G495-11 was performed in a vegetative medium for 4–5 days at 28 °C on a rotary shaker. The broth (128 L) was separated into mycelial cake and supernate. The mycelial cake was extracted with methanol, and the extract was combined with the supernate. The combined liquid was extracted twice with EtOAc at pH 2.0, and the EtOAc layer was shaken with dilute NaOH solution (35 L). The alkaline solution was acidified to pH 2.0 with 6 M hydrochloric acid to yield a crude solid (24 g). Chromatographic separation on silica gel eluted by $CHCl_3$/MeOH/AcOH (20:5:1) afforded boxazomycin A (5.7% of the crude solid) and B (0.9%), after recrystallization from methanol.

2-[5'-Hydroxy-2'-(hydroxymethyl)pyrimidin-4'-yl]-5-hydroxy-7-methylbenzoxazole-4-carboxamide (Boxazomycin A) (1) and 2-(5'-Hydroxy-2'-methylpyrimidin-4'-yl)-5-hydroxy-7-methylbenzoxazole-4-carboxamide (Boxazomycin B) (2). Boxazomycin A (**1**): pale yellow needles; mp >275 °C dec MeOH; MS m/e 316 (M^+), 299, 215; ^1H NMR (60 MHz, D_2O/NaOD) δ 2.35 (3 H, s), 4.47 (2 H, s), 6.43 (1 H, s), 7.93 (1 H, s); UV [EtOH/DMF (95:5)] λ_{max} (log ϵ) 364 nm (4.13), 379 (sh, 4.10), 420 (sh, 3.79); UV (0.1 M NaOH) λ_{max} (log ϵ) 219 nm (4.46), 230 (sh, 4.39), 259 (sh, 4.12), 403 (4.30); IR (KBr) 3450, 3350, 3200, 3050, 1650, 1625, 1595, 1560, 1535, 1505 cm^{-1}. Anal. Calcd for $C_{14}H_{12}N_4O_5 \cdot {}^1/_2H_2O$: C, 52.41; H, 4.22; N, 16.87. Found: C, 52.80; H, 4.21; N, 16.59. Boxazomycin B (**2**): pale yellow powder; mp >270 °C dec (MeOH); MS m/e 300 (M^+), 283, 215; ^1H NMR (60 MHz, D_2O/NaOD) δ 2.20 (3 H, s), 2.30 (3 H, s), 6.45 (1 H, s), 7.80 (1 H, s); UV [EtOH/DMF (95:5)] λ_{max} (log ϵ) 364 nm (4.17), 379 (sh, 4.38). 420 (sh, 3.34); UV (0.1 M NaOH) λ_{max} (log ϵ) 219 nm (4.46), 230 (sh, 4.38), 259 (sh, 4.06), 403 (4.32); IR (KBr) 3410, 3310, 3200, 3050, 1665, 1625, 1600, 1560, 1530, 1510 cm^{-1}. Anal. Calcd for $C_{14}H_{12}N_4O_4$: C, 56.00; H, 4.03; N, 18.66. Found: C, 55.63; H, 3.82; N, 18.34.

Acetylation of Crude Boxazomycin A (1). A solution of crude boxazomycin A **1**; 1.02 g, 3.2 mmol) in acetic anhydride (10 mL) and pyridine (10 mL) was stirred at room temperature for 48 h. The solution was concentrated to a solid residue under a reduced pressure (oil pump) at room temperature. The solid was chromatographed (flash chromatography) with EtOAc/CH_2Cl_2 (2:3) as an elution solvent to afford solid **4** (1.1 g). Recrystallization from benzene gave rise to pure **4** as silky needles: mp 185 °C; MS m/e 484 (M^+), 442, 400, 358, 316, 298, 281; UV (MeOH) λ_{max} (log ϵ) 207 nm (4.63), 280 (sh, 4.08), 320 (4.42); IR (KBr) 3410, 3270, 2980, 2940, 1770, 1755, 1745, 1715, 1690, 1630, 1565, 1540, 1505 cm^{-1}. Anal. Calcd for $C_{22}H_{20}N_4O_9$: C, 54.39; H, 4.19; N, 11.52. Found: C, 54.54; H, 4.39; N, 11.56. Elution with EtOAc gave 2-[5'-acetoxy-2'-(acetoxymethyl)pyrimidin-4'-yl]-7-methylbenzoxazole-

(20) This effect results from the ^{14}N nuclear quadrupole moment interfering with the ability of magic angle spinning to average out carbon–nitrogen dipolar coupling. See: Lippmaa, E.; Alla, M.; Kendla, E. 18th Experimental NMR Conference, Asilomar, CA, 1977. Frey, M. H.; Opella, S. J. *J. Chem. Soc., Chem. Commun.* **1980**, 474. Harris, R. K.; Jonsen, P.; Packer, K. J. *Magn. Reson. Chem.* **1985**, *23*, 565.

(21) Brockmann, H.; Muxfeldt, H. *Chem. Ber.* **1958**, *91*, 1242.

4-carboxamide (diacetylboxazomycin C) (**6**): 137 mg; pink silky needles; mp 230–231 °C (benzene; MS *m/e* 384 (M^+), 342, 300, 283, 282, 267, 266, 255, 196; ^1H NMR (90 MHz, DMSO-d_6) δ 2.28 (3 H, s), 2.55 (3 H, s), 2.69 (3 H, s), 5.40 (2 H, s), 7.58 (1 H, d, J = 8.0 Hz), 7.98 (1 H, d, J = 8 Hz), 8.09 (2 H, br s), 9.13 (1 H, s); UV (MeOH) λ_{max} (log ϵ) 224 nm (4.20), 252 (4.01), 318 (4.21); IR (KBr) 3390, 3180, 2940, 2870, 1765, 1755, 1645, 1625, 1570, 1560, 1545 cm^{-1}. Anal. Calcd for $C_{18}H_{16}N_4O_6$: C, 56.25 H, 4.19; N, 14.57. Found: C, 56.25; H, 4.17; N, 14.44. The ^{13}C NMR of **6** could not be obtained because of poor solubility. Therefore, **6** was converted into more soluble formimide **10** by the following procedure. Diacetylboxazomycin C (34.3 mg, 0.89 mmol) was heated at 120 °C with dimethylformamide dimethyl acetal (0.5 mL, 3.8 mmol) for 75 min. The excess reagent was evaporated under a reduced pressure, and 70% AcOH (2 mL) was added onto the residue. The mixture was stirred at room temperature for 15 min, and the acetic acid was removed on a rotary evaporator. The resulting solid was recrystallized from benzene to afford *N*-formyl-2-[2′-(acetoxymethyl)-5′-methoxypyrimidin-4′-yl]-7-methylbenzoxazole-4-carboxamide (**10**) as brown flakes: mp 231–233 °C; MS *m/e* 384 (M^+), 342; UV (MeOH) λ_{max} (log ϵ) 234 nm (4.17), 282 (3.87), 324 (4.02), 341 (4.13), 358 (4.00); IR (KBr) 3460, 3260, 2950, 2860, 1745, 1725, 1680, 1635, 1595, 1565, 1545, 1520, 1510 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) δ 2.25 (3 H, s), 2.73 (3 H, s), 4.22 (3 H, s), 5.50 (2 H, s), 7.45 (1 H, d, J = 8 Hz), 8.74 (1 H, s), 9.57 (1 H, d, J = 11 Hz), 11.7 (1 H, br d, J = 11 Hz); ^{13}C NMR (22.5 MHz, DMSO-d_6) δ 15.8 (q, C-10), 20.9 (q, 7′-OAc), 57.1 (q, 5′-OMe), 65.7 (t, C-7′), 119.6 (s, C-4), 127.2 (d, C-5), 128.2 (d, C-6), 128.7 (s, C-7), 139.3 (s, C-4′ or -3a), 139.6 (s, C-3a or -4′), 143.8 (d, C-6′), 149.4 (s, C-5′ or -7a), 151.6 (s, C-7a or -5′), 157.2 (s, C-2′), 158.4 (s, C-2), 162.9 (d, N_9 CHO), 164.1 (s, C-8), 170.7 (s, 7′-OAc). Anal. Calcd for $C_{18}H_{16}N_4O_6$: C, 56.25; H, 4.19; N, 14.57. Found: C, 56.33; H, 4.24; N, 14.39.

Acetylation of Boxazomycin B (2). A solution of crude boxazomycin B (**2**; 80.5 mg) in acetic anhydride (0.8 mL) and pyridine (0.8 mL) was stirred at room temperature for 48 h. The solution was concentrated to a solid residue under a reduced pressure. The solid was chromatographed on silica gel (Wakogel C-300) with AcOEt/CH_2Cl_2 (2:3) as an elution solvent to afford solid acetate **5**:12 mg; light brown amorphous; mp 187–191 °C; UV (MeOH) λ_{max} (log ϵ) 202 nm (4.69), 280 (sh, 4.15), 316 (4.39); IR ($CHCl_3$) 3270, 2950, 2850, 1760, 1740, 1715, 1690, 1650, 1630, 1570 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.43 (3 H, s, 5-OAc), 2.48 (3 H, s, 5′-OAc), 2.60 (3 H, s, 9-Ac), 2.71 (3 H, s, 10-H_3), 2.90 (3 H, s, 2′-Me), 7.11 (1 H, s, 6-H), 8.71 (1 H, s, 6′-H), 11.05 (1 H, s, 9-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 15.8 (C-10), 20.9 (5′-OAc), 25.6 (9-Ac), 26.3 (7′-Me), 113.2 (C-4), 125.0 (C-6), 128.7 (C-7), 139.6 (C-3a), 142.1 (C-5′), 143.6 (C-4′), 147.2 (C-7a), 149.9 (C-5), 154.3 (C-6′), 158.8 (C-2), 160.8 (C-8), 166.2 (C-2′), 168.9 (5′-OAc), 170.0 (5-OAc), 173.1 (9-Ac).

Phosphorylation of Boxazomycin A (1). A mixture of **1** (205 mg, 0.65 mmol), diethyl chlorophosphate (2.4 mL, 17 mmol), and 2,6-dimethylpyridine (2 mL, 17 mmol) was stirred at room temperature for 18 h. The mixture was poured onto 0.05 M hydrochloric acid (300 mL) and extracted with Et_2O (300 mL) twice. The combined ethereal layer was washed with 0.02 M hydrochloric acid (300 mL) three times and dried over Na_2SO_4. Evaporation of the solvent afforded an oil (315 mg), which was chromatographed [flash chromatography; EtOAc/acetone (7:1)] to give the diphosphate **7** (62 mg) as a yellow solid. The material was pure on TLC [EtOAc/acetone (7:1)]: UV (MeOH) λ_{max} (log ϵ) 236 nm (sh, 4.10), 316 (4.00), 354 (4.50); IR (KBr) 3400, 3230, 3000, 2950, 1660, 1640, 1570, 1520 cm^{-1}; ^1H NMR (60 MHz, $CDCl_3$) δ 1.30 (6 H, t, J = 8 Hz), 1.34 (6 H, t, J = 8 Hz), 2.57 (3 H, s), 4.20 (8 H, quintet, J_{HH} = 8 Hz, J_{PH} = 8 Hz), 5.33 (2 H, d, J = 9 Hz), 6.05 (1 H, br s), 6.90 (1 H, s), 8.90 (1 H, br s), 8.97 (1 H, s), 12.90 (1 H, s); ^{13}C NMR (22.5 MHz, $CDCl_3$; multiplicity and J are for a long-range coupling between carbon and phosphorus atoms) δ 15.3 (C-10), 15.6 (d, J = 6.7 Hz, $POCH_2$*Me*), 15.7 (d, J = 6.4 Hz, $POCH_2$*Me*), 64.0 (d, J = 5.9 Hz), 65.4 (d, J = 6.0 Hz), 68.0 (t, J = 5.0 Hz, C-7′) 101.5 (C-4), 118.3 (C-6), 128.1 (C-7), 136.9 (C-3a), 142.8 (C-7a), 142.9 (d, J = 6.3 Hz, c-4′ or -5′), 143.0 (d, J = 7.2 Hz, C-5′ or -4′), 151.9 (d, J = 2.5 Hz, C-6′), 156.7 (d, J = 1.3 Hz, C-2), 161.1 (dd, J = 7.5, 1.1 Hz, C-2′), 163.1 (C-5), 170.7 (C-8).

***N*-Acetyl-2-[5′-methoxy-2′-(acetoxymethyl)pyrimidin-4′-yl]-5-acetoxy-7-methylbenzoxazole-4-carboxamide (8).** A solution of boxazomycin A (**1**; 100.5 mg, 0.32 mol) in DMF (50 mL) was treated with an excess ethereal solution of diazomethane. The mixture was stirred at room temperature for 25 h, and the ether was removed on a rotary evaporator. Then, the DMF was evaporated at 55 °C by use of an oil pump, and Ac_2O (1.7 mL) and pyridine were added. The mixture was stirred at room temperature for 24 h. The solvent and reagent were evaporated under a reduced pressure, and the residue was purified by flash chromatography [EtOAc/CH_2Cl_2 (1:1)] to give **8**:21 mg, 14%; mp 194–195 °C (benzene/hexane); MS *m/e* 456 (M^+), 414, 400, 373, 355, 312, 298; UV (MeOH) λ_{max} (log ϵ) 234 nm (sh, 4.38), 282 (4.07), 345 (4.36), 361 (sh, 4.25); IR (KBr) 3450, 3260, 3200, 3000, 2950, 2850, 1760, 1740, 1720, 1690, 1640, 1590, 1580, 1520 cm^{-1}; ^1H NMR (60 MHz, $CDCl_3$) δ 2.25 (3 H, s), 2.42 (3 H, s), 2.60 (3 H, s), 2.69 (3 H, s), 4.25 (2 H, s), 7.05 (1 H, s), 8.71 (1 H, s), 11.92 (1 H, br s); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 15.7 (q, C-10), 20.9 (q, 7′- or 5-OAc), 21.1 (q, 5- or 7′-OAc), 26.2 (q, N_9 Ac), 56.9 (q, 5-OMe), 65.7 (t, C-7′), 112.5 (s, C-4), 124.7 (d, C-6), 128.4 (s, C-7), 138.7 (s, C-4′), 140.0 (s, C-3a), 143.7 (d, C-6′), 146.7 (s, C-8), 149.8 (s, C-5), 151.6 (s, C-5′), 156.8 (s, C-2′), 158.5 (s, C-2), 160.8 (s, C-8), 169.9 (s, 5-OAc), 170.7 (s, 7′-OAc), 173.2 (s, N_9 Ac). Anal. Calcd for $C_{21}H_{20}H_4O_8$: C, 55.26; H, 4.41; N, 12.27. Found: C, 54.91; H, 4.37; N, 12.13. Also an unexpected product, *N*-formyl-2-[5′-methoxy-2′-(acetoxymethyl)pyrimidin-4′-yl]-5-acetoxy-7-methylbenzoxazole-4-carboxamide **9**; 48 mg, 36%) was obtained as an amorphous powder [mp 198–200 °C (MeOH)]; MS *m/e* 414 (M^+ – C=O), 400, 372, 355, 329, 330, 313, 312, 298; UV (MeOH) λ_{max} (log ϵ) 362 nm (sh, 4.26), 347 (4.35), 282 (4.09), 236 (4.38); IR (KBr) 3400, 3260, 3200, 2950, 1770, 1750, 1730, 1690, 1640, 1590, 1580, 1520, 1510 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) δ 2.24 (3 H, s), 2.41 (3 H, s), 2.70 (3 H, s), 4.24 (3 H, s), 5.40 (2 H, s), 7.09 (1 H, s), 8.74 (1 H, s), 9.40 (1 H, d, J = 10 Hz), 12.0 (1 H, br d, J = 10 Hz); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 15.5 (q, 10-Me), 20.8 (q, Ac), 21.0 (q, Ac), 56.9 (q, 5′-OMe), 65.5 (t, C-7′), 111.2 (s, C-4), 124.5 (d, C-6), 129.2 (s, C-7), 138.4 (s, C-4′), 140.2 (s, C-3a), 143.7 (d, C-6′), 146.7 (s, C-7a), 149.8 (s, C-5), 151.4 (s, C-5′), 156.8 (s, C-2′), 158.7 (s, C-2), 160.2 (s, C-8), 162.7 (d, N_9 CHO), 169.6 (s, 5-OAc), 170.5 (s, 7′-OAc). Anal. Calcd for $C_{20}H_{18}N_4O_8$: C, 54.05; H, 4.06; N, 12.41. Found: C, 54.30; H, 4.10; N, 12.67.

Methyl 2-Methyl-5-methoxypyrimidine-4-carboxylate (11). A mixture of 5-bromo-2-methylpyrimidine-4-carboxylic acid[22] (44 mg, 0.2 mmol), 0.55 M methanolic NaOMe (0.8 mL, 0.44 mmol), and $Cu^{II}O$ (3 mg) was heated at 120 °C under argon in a sealed tube for 18 h. After the tube was opened and the mixture was neutralized with concentrated hydrochloric acid, the solid was removed by filtration with the aid of MeOH. The filtrate was concentrated, and water (50 mL) was added. The product was taken up in EtOAc (100 mL), and the organic layer was dried over Na_2SO_4. Concentration of the solution afforded crude acid (30 mg), which was methylated with diazomethane by the usual method. The crude methyl ester was purified by sublimation [80–100 °C (20 mm)] to give pure **11**: 9.5 mg, 26%; colorless needles; mp 47–48 °C; MS 182 (M^+), 152, 123, 94, 83; UV (MeOH) λ_{max} (log ϵ) 219 nm (4.03), 288 (3.38); IR (KBr) 3000, 2950, 1720, 1570 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.74 (3 H, s), 3.99 (6 H, s), 8.50 (1 H, s); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 24.2 (q, 2-Me), 52.5 (q, CO_2Me), 56.5 (q, 5-OMe), 142.8 (d, C-6), 145.2 (s, C-4), 148.8 (s, C-5), 159.7 (s, C-2), 163.9 (s, CO). Anal. Calcd for $C_8H_{10}N_2O_3$: C, 52.69; H, 5.54; N, 15.44. Found: C, 52.74; H, 5.53; N, 15.37.

Acknowledgment. We are grateful to Bristol-Meyers Research Institute, Tokyo, for a donation of the samples of boxazomycins.

(22) Buděšínský, Z. *Collect. Czech. Chem. Commun.* **1949**, *14*, 223.