4.70 (1 H, d, J = 5.5 Hz), 4.57 (1 H, dm, J_{P-H} = 41.8 Hz), 3.27 (1 H, d quint, J_{P-H} = 20.9 Hz, J_{H-H} = 6.0 Hz), 3.08 (3 H, d, J = 8.5 Hz), 2.76 (2 H, dm, J_{P-H} = 23.8 Hz), 1.70 (3 H, d, J = 1.2 Hz), 0.70 (3 H, d, J = 6.3 Hz); ^{13}C NMR ($CDCl_3$) 152.0 (d, J_{P-C} = 13.3 Hz), 141.3 (d, J_{P-C} = 168.2 Hz), 140.0 (d, J_{P-C} = 6.2 Hz), 130.3 (d, J_{P-C} = 10.5 Hz), 128.0, 127.9, 127.7, 127.5, 126.1 (d, J_{P-C} = 74.9 Hz), 125.7, 72.7, 59.8 (d, J_{P-C} = 13.8 Hz), 34.9 (d, J_{P-C} = 4.9 Hz), 30.2 (d, J_{P-C} = 124.6 Hz), 17.2 (d, J_{P-C} = 2.6 Hz), 14.6; ^{31}P NMR ($CDCl_3$) –27.23 ppm; IR ($CHCl_3$, cm^{-1}) 1461 (C=C), 1097 (PPh), 1014 (PO); $[\alpha]_D$ +6.1° (c = 0.41, $CHCl_3$); exact mass calcd for $C_{20}H_{24}NO_4P$ $(M)^+$ 341.1543, found 341.1532.

(4*S*,5*R*)-2-(4-Methoxyphenyl)-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholane (5b). The preparation was carried as for the preparation of **5a**[18] using the following quantities of reagents: (–)-ephedrine (0.610 g, 3.7 mmol), Et_3N (0.747 g, 7.38 mmol), and dichloro(4-methoxyphenyl)phosphine[25] (0.772 g, 3.7 mmol): yield 64% (0.715 g); mp 85 °C dec; ^{1}H NMR ($CDCl_3$) 7.46 (2 H, dd, J_{P-H} = 10.6 Hz, J_{H-H} = 5.4 Hz), 7.4–7.2 (5 H, m), 6.96 (2 H, d, J = 8.30 Hz), 5.53 (1 H, d, J = 6.8 Hz), 3.83 (3 H, s), 3.30 (1 H, quint d, J_{H-H} = 6.6 Hz, J_{P-H} = 2.4 Hz), 2.52 (3 H, d, J = 13.8 Hz), 0.68 (3 H, d, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$) 160.6, 139.2, 133.6 (d, J_{P-C} = 49.5 Hz), 131.2 (d, J_{P-C} = 21.3 Hz), 127.9, 127.6, 127.2, 113.6 (d, J_{P-C} = 5.5 Hz), 85.9 (d, J_{P-C} = 9.3 Hz), 56.6 (d, J_{P-C} = 5.6 Hz), 55.1, 30.0 (d, J_{P-C} = 8.6 Hz), 13.7 (d, J_{P-C} = 3.6 Hz); ^{31}P NMR ($CDCl_3$) +144.23; IR ($CDCl_3$, cm^{-1}) 1094 (PPh), 975 (PO); $[\alpha]_D$ = –11.2° (c = 0.25, $CHCl_3$); exact mass calcd for $C_{17}H_{20}NO_2P$ $(M)^+$ 301.1230, found 301.1211.

Acknowledgment. We would like to thank the donors of the Petroleum Research Fund, administered by the American Chemical Society, and the University of Delaware Research Foundation for support of this research.

Supplementary Material Available: Experimental details and spectral data for compounds **1a**, **2a**, **3a**, **4**, and **5b** and ^{1}H NMR spectra of compounds **1a**, **2a**, **3a**, and **4** (8 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

The Structures of A10255B, -G, and -J: New Thiopeptide Antibiotics Produced by *Streptomyces gardneri*

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The structures of the major members of a new family of important thiopeptide antibiotics, A10255B (**1**), A10255G (**15**), and A10255J (**16**), produced by *Streptomyces gardneri* (NRRL 15537), are described. Selective chemical degradation in combination with NMR, FABMS, and CID methods on the degradation products was required to solve these structures. Methanolysis of **1** resulted in the isolation of 4-carbomethoxy-2-propionyloxazole (**8**) and dimethyl sulfomycinamate (**9**) as well as *N*-((acetamidomethyl)thiazolyl)-1-(carbomethoxythiazolyl)ethanamide (**11**) after acetylation. Vigorous treatment with acid produced berninamycinic acid (**10**). Trifluoroacetolysis led to cleavage at the six dehydroalanine (deala) residues to give a complex and highly modified pentapeptide **12** which was sequenced by CIDMS and NMR techniques. Compound **12** was composed of the following: sulfomycinamic acid, threonine, 1-(4-carboxyoxazolyl)-1-aminopentene unit (dehydronorvaline masked by oxazole at its carboxyl group), 2-(aminomethyl)thiazole-4-carboxylic acid, and 2-(1-aminoethyl)-4-carboxamidothiazole. FABMS and base hydrolysis showed that **1** had a deala tetrapeptide side chain. Antibiotics **15** and **16** each had a masked dehydrobutyrine in place of the dehydronorvaline present in **1**, and **16** had a single amidated deala as a side chain.

Introduction

A10255, a novel sulfur-containing complex of antibiotics produced by *Streptomyces gardneri*, exhibits strong antimicrobial activity against Gram-positive bacteria and has potential utility as a growth promotant and as a preventative of lactic acidosis in farm animals.[1,2] The A10255 complex was extracted from the mycelia formed in submerged cultures of the producing organism and shown to be multicomponent (designated A10255B, -C, -E, -F, -G, -H, and -J) by chromatography. The major components A10255B, -J, and -G were isolated in sufficent quantity to permit determination of their structures.

Physicochemical data indicated that the A10255 antibiotics belong to the thiopeptide class. Members of this class characteristically possess a cyclic peptide core composed mostly of amino acids masked at their carboxyl groups by thiazole and/or oxazole rings as well as the presence of several dehydroamino acids. These antibiotics have presented chemists with formidable structure elucidation tasks. In the study reported here the most direct approaches were not available to the solution of the structures of the A10255 antibiotics. The noncrystalline nature of these substances precluded X-ray crystallographic work. This paper presents a chemical degradation scheme which led to the solution of this problem and the elucidation of the structures of the major components of this family of antibiotics: A10255B, A10255G, and A10255J.

† Part of this work has been presented at the 29th Interscience Conference on Antimicrobial Agents and Chemotherapy, Houston TX, Sept 17, 1989; Abstract 410.

(1) Richardson, L. F.; Scheifinger, C. C.; Becker, D. A. Abstracts of the 29th Interscience Conference on Antimicrobial Agents and Chemotherapy, Houston, TX, Sept 17, 1989; Paper 412; Am. Soc. Microbiol., Washington, D.C. 20006.

(2) Michel, K.; Hoehn, M. M.; Boeck, L. D.; Martin, J. W.; Abbott, M. A.; Godfrey, O. W.; Mertz, F. P. Abstracts of the 29th Interscience Conference on Antimicrobial Agents and Chemotherapy, Houston, TX, Sept 17, 1989; paper 409; Am. Soc. Microbiol., Washington, D.C. 20006.

0022-3263/92/1957-5200$03.00/0

Table I. ^{13}C-NMR Data for 1, 15, and 16 in DMSO-d_6[a]

position	1	15	16
oxazole (1)			
2	158.10	158.01	158.02
4	139.23	139.20	139.14
5	140.68	140.62	140.25
pyridine (2)			
2	146.88	146.83	146.70
3	130.21	130.14	130.09
4	141.32	141.26	141.17
5	121.47	121.40	121.28
6	149.37	149.41	149.63
6-C=O	161.57	161.52	161.10
thiazole (3)			
2	163.67	163.59	163.52
4	148.79	149.41	149.39
5	127.22	127.08	127.06
4-C=O	160.17	160.12	160.07
threonine			
CHN	57.94	57.94	57.92
CHO	67.37	67.26	67.23
CH_3	20.32	20.22	20.21
C=O	169.17	168.89	168.86
dehydronorvaline			
C=	122.36		
CH=	135.26		
CH_2	20.74		
CH_3	12.93		
dehydrobutyrine			
C=		123.66	123.65
CH=		128.70	128.63
CH_3		13.41	13.39
oxazole (4)			
2	159.46	159.42	159.38
4	136.14	136.04	136.06
5	142.05	141.94	141.89
4-C=O	160.32	160.24	160.20
thiazole (5)			
2	168.87	168.71	168.68
4	148.79	148.73	148.70
5	124.98	124.92	124.87
4-C=O	160.01	159.94	159.92
2-CH_2	40.24	40.12	40.16
thiazole (6)			
2	172.88	172.80	172.80
4	148.05	148.04	148.00
5	125.94	125.83	125.78
4-C=O	158.96	158.91	158.86
2-CH	46.53	46.49	46.50
CH_3	20.74	20.60	20.59
dehydroalanine			
C=	136.33	136.25	133.76
	136.29	136.20	133.76
	134.23	134.20	128.96
	133.82	133.81	
	133.28	133.20	
	129.07	128.99	
CH_2=	110.98	110.77	110.25
	110.92	110.22	104.62
	110.49	109.85	103.00
	109.46	109.35	
	106.23	106.12	
	104.73	104.65	
C=O	164.90	164.80	164.93
	163.07	163.00	162.58
	162.84	162.79	
	162.68	162.61	
	162.61	162.53	

[a] Temperature = 330 K.

Table II. ^{1}H-NMR Data for 1, 15, and 16 in DMSO-d_6[a]

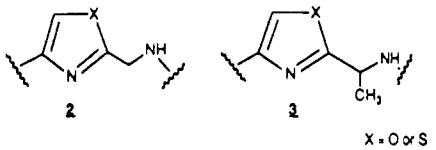
position	1	15	16
oxazole (OXZ 1)			
5	8.67 (s)[e]	8.68 (s)	8.60[d] (s)
pyridine (PYR 2)			
4	8.48 (d)	8.48 (d)	8.49 (s)
5	8.25 (d)	8.24 (d)	8.25 (d)
thiazole (THZ 3)			
5	8.51 (s)	8.51 (s)	8.51 (s)
threonine			
CHN	4.63 (dd)	4.63 (dd)	4.63 (dd)
CHO	4.24 (d,q)	4.24 (d,q)	4.25 (d,q)
CH_3	1.11 (d)	1.11 (d)	1.12 (d)
dehydronorvaline			
CH=	6.39 (t)		
CH_2	2.21 (dq)		
CH_3	1.02 (t)		
dehydrobutyrine			
CH=		6.47 (q)	6.48 (q)
CH_3		1.77 (d)	1.78 (d)
oxazole (OXZ 4)			
5	8.60 (s)	8.60 (s)	8.58 (s)
thiazole (THZ 5)			
5	8.24 (s)	8.24 (s)	8.25 (s)
2-CH_2	4.80/4.67 (dd/dd)	4.80/4.67 (dd/dd)	4.80/4.67 (dd/dd)
thiazole (THZ 6)			
5	8.39 (s)	8.39 (s)	8.39 (s)
2-CH	5.44 (dq)	5.44 (dq)	5.44 (dq)
CH_3	2.21 (d)	1.62 (d)	1.62 (d)
dehydroalanine			
CH_2=	5.82/5.76[e]	5.81/5.76[b]	5.80[c]/5.76
	5.79/5.64	5.80/5.64	5.80[c]/5.66
	6.11/5.76	6.10/5.76[b]	6.55/5.82[d]
	5.84/5.68	5.84/5.68	
	6.53/5.95	6.53/5.95	
	6.53/5.76	6.53/5.78[b]	
exchangeables			
	10.53	10.53	10.69
	9.95	9.95	9.86
	9.86	9.83	9.80
	9.79	9.79	9.58
	9.61	9.59	8.87 (t)
	9.58	9.59	8.84 (d)
	9.09	9.09	8.15
	8.90 (t)	8.89 (t)	8.04 (d)
	8.84 (d)	8.84 (d)	7.65
	8.04 (d)	8.04 (d)	5.16 (d)
	5.16 (d)	5.16 (d)	

[a] Temperature = 330 K. [b-d] Assignments with the same superscript may be interchanged. [e] All dehydroalanine resonances and nonlabeled exchangeables are singlets.

Results and Discussion

A10255B (1) was obtained as a pure noncrystalline solid whose elemental composition was determined by high-resolution mass spectrometry (HRMS) ($C_{53}H_{48}N_{16}O_{15}S_3$ m/z 1245 (MH^+)). The ^{13}C-NMR spectrum revealed the presence of 53 carbons while the ^{1}H-NMR showed 47 hydrogens, 11 of which were exchangeable (Tables I and II).

^{1}H-NMR also showed the presence of a butenyl group, 12 additional olefinic protons, and two masked amino acids: thiazole (or oxazole)-gly (2) and thiazole (or oxazole)-ala (3). The ^{13}C NMR ID and DEPT data show that the 12



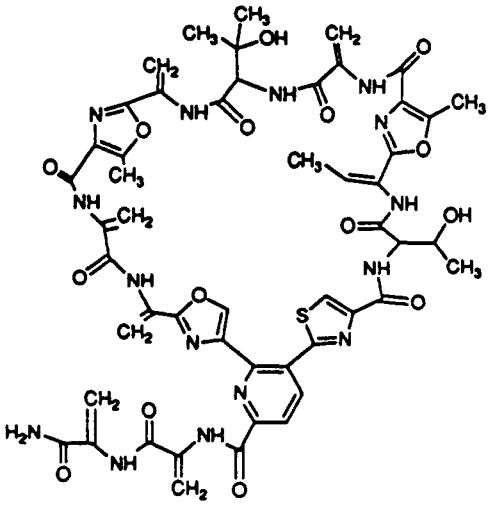
2 3

X = O or S

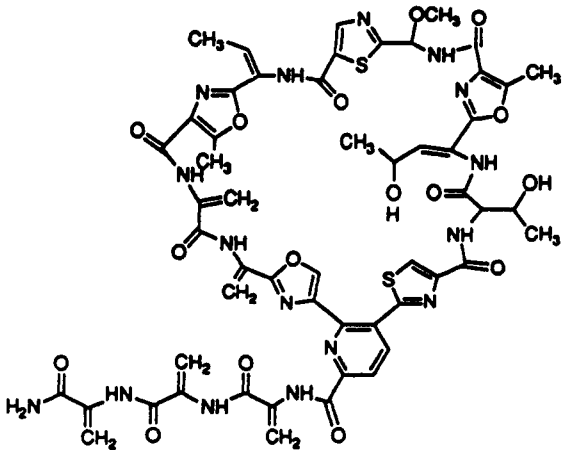
^{1}H olefinic resonances represent six dehydro (or masked dehydro) alanines. The IR and NMR data indicated that 1 was a peptide. Potentiometric titration (66% DMF) revealed an acidic group (pK_a = 4.4) and a basic group (pK_a = 11.1). The nature of these data resembled that of the highly modified class of thiopeptide antibiotics which includes berninamycin A (4),[3,4] sulfomycin I (5),[4] thioxamycin (6),[5] nosiheptide,[6] thiopeptin,[7] and thiostrepton[8] and served

(3) Liesch, J. M.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1977**, *99*, 1645.
(4) Abe, H.; Kushida, K.; Shiobara, Y.; Kodama, M. *Tetrahedron Lett.* **1988**, 1401.

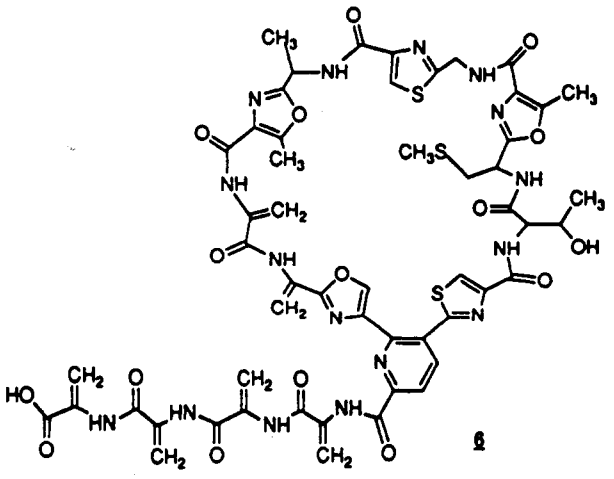
to identify A10255B as a new member of this class. 2D long-range $^1H/^{13}C$ correlation NMR experiments did not provide sufficient data to determine the complete structure of 1, and chemical degradation experiments were undertaken in order to provide more structural information of fragments and how they were connected.



4



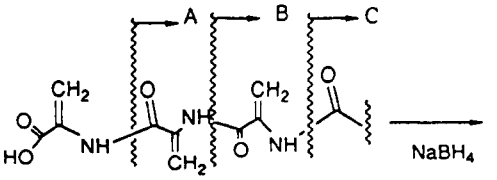
5



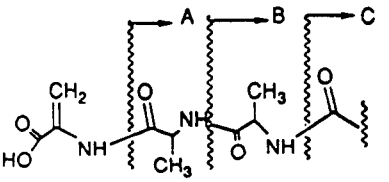
6

Sodium Borohydride Reduction. Amino acid analysis of 1 showed a single threonine residue, a number of unidentified amino acids, and the evolution of several moles of ammonia, a characteristic of the hydrolysis of dehydroamino acids.[9] The presence of dehydroamino acids was verified by reduction of 1 with $NaBH_4$ which gave reduction product 7, the latter releasing five alanine residues and a single norvaline upon amino acid analysis.[10] FABMS of 7 showed an MH^+ ion at m/z 1257, reflecting a net gain of 12 hydrogens upon reduction. ^1H-NMR spectra revealed that a dehydroalanine (deala) residue remained unreduced (signals for olefinic protons at 5.69 and 6.26 ppm). It was concluded that a total of six deala residues (or their equivalent) and a dehydronorvaline moiety were present in 1.

The unimolecular dissociation spectrum of the protonated molecular ion of the $NaBH_4$ reduction product of A10255B (7) (m/z 1257) showed a series of fragmentations at m/z 1170, 1099, and 1028. The initial fragment A in each case corresponded to the loss of 87 mass units and indicated that the C-terminal amino acid in this reduction product was an unreduced deala. The remaining two fragments B and C in the unimolecular dissociation spectrum of 7 correspond to the sequential loss of 71 mass units (alanyl groups) in agreement with the sequence shown in eq 1. The unimolecular dissociation spectrum



(1)



(eq. 1)

(7)

of the protonated molecular ion of 1, m/z 1245 (MH^+), had prominent fragments at m/z 1158, 1089, and 1020 which were used to assign the position of three of the five deala groups. These data correspond to the sequential loss of three deala units from the C-terminus to yield a series of b-type peptide fragments designated as A, B, and C in eq 1.

Methanolysis. Methanolysis of 1 gave a mixture of degradation products (see Scheme I). Two of these were isolated by solvent extraction and chromatographic separation: 4-carbomethoxy-2-propionyloxazole (8) and 9, whose properties matched those of dimethyl sulfomycinamate, isolated previously from sulfomycin I (5) by Abe.[11]

(5) Matsumoto, M.; Kawamura, Y.; Yasuda, Y.; Tanimoto, T.; Matsumoto, K.; Yoshida, T.; Shoji, J. *J. Antibiot.* **1989**, *42*, 1465. Although this reference contains the structure of thioxamycin and refers to a publication in preparation for the documentation for its proposed structure, the latter paper has not yet been published.

(6) Depaire, H.; Thomas, J. P.; Brun, A.; Olesker, A.; Lukacs, G. *Tetrahedron Lett.* **1977**, 1403.

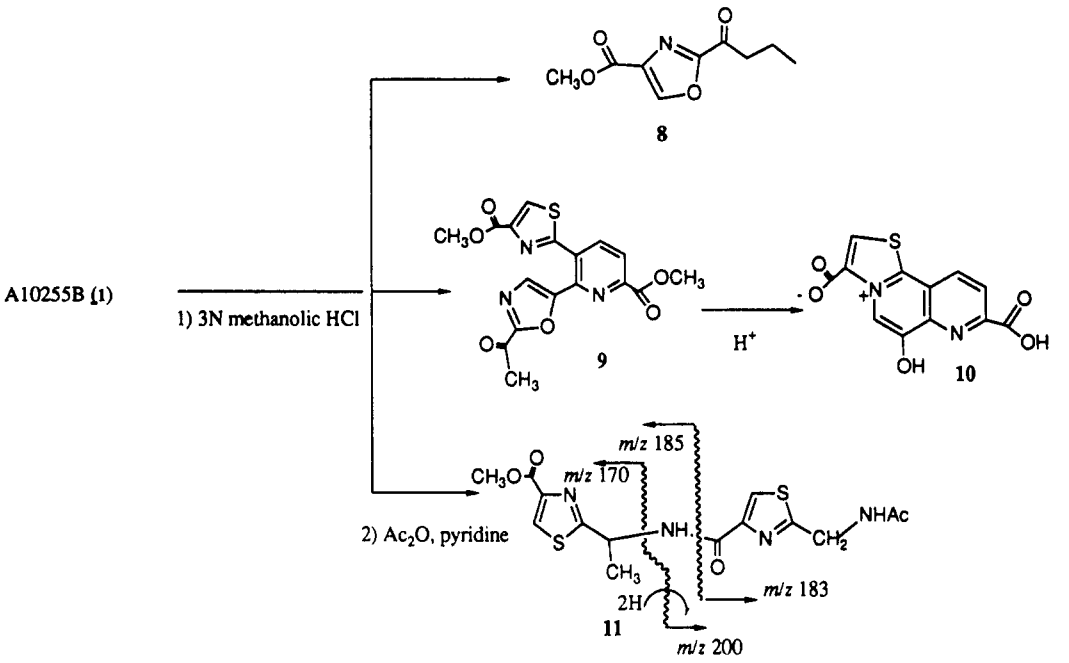
(7) Hensens, O. D.; Albers-Schonberg, G. *Tetrahedron Lett.* **1978**, 3649.

(8) (a) Hensens, O. D.; Albers-Schonberg, G. *J. Antibiot.* **1983**, *36*, 832. (b) Hensens, O. D.; Albers-Schonberg, G.; Anderson, B. F. *J. Antibiot.* **1983**, *734*, 799. (c) Tori, K.; Takura, K.; Okabe, K.; Ebata, M.; Otsuka, H.; Lukacs, G. *Tetrahedron Lett.* **1976**, 185. (d) Anderson, B.; Hodgkin, D. C.; Viswamitra, M. A. *Nature* **1970**, *25*, 233.

(9) Liesch, J. M.; Millington, D. S.; Pandey, R. C.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1976**, *98*, 8237.

(10) Bodansky, M.; Scozzi, J. A.; Muramatzu, I. *J. Antibiot.* **1970**, *23*, 9.

Scheme I

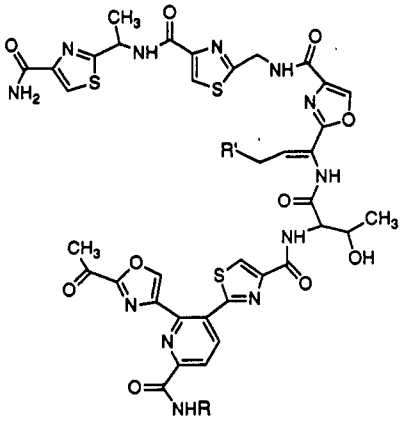


Berninamycinic acid (**10**), a degradation product arising from acidic hydrolysis of sulfomycinamic acid and **4**, was isolated upon extended acid hydrolysis of **1**.[16] Abe et al. have reported that the isolation of **10** under these conditions is characteristic of thiopeptides containing a sulfomycinamic acid moiety.[4,11] Oxazole **8** was likely formed from the masked dehydronorvaline residue, a transformation that had been noted by other workers in studies of berninamycin A[9] and nosiheptide.[15]

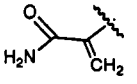
The mixture of water-soluble products from the methanolysis reaction was lyophilized and acetylated, and a further product, **11**, was isolated. The ^{1}H-NMR spectrum of **11** showed the presence of alanine and glycine residues, masked at their carboxyls as thiazoles which were also evident in the ^{1}H-NMR spectrum of **1**. The structure of this fragment was assigned on the basis of the collisionally induced dissociation mass spectrometry (CIDMS) pattern shown in structure **11** and NMR and mass spectral studies on larger fragments of **1**.

Trifluoroacetolysis. Dehydroamino acids are known to cleave selectively under acidic or basic conditions.[6,10,12] A10255B (**1**) was treated with anhydrous trifluoroacetic acid, and the resulting mixture was purified to give major product **12**. The elemental composition of **12** corresponded to the removal of five deala residues from **1**. A minor product with one additional deala unit (**13**) was also isolated from this reaction mixture. In **1**, the sulfomycinamic acid moiety likely serves to bridge the peptide ring with a masked dehydroalanine as part of its oxazole ring. This deala residue is converted to the methylketone group under trifluoroacetolysis as it was in conventional aqueous acid treatment. Therefore, the net loss of five deala residues does not include the residue embedded in the sulfomycinamic acid group.

The amino acid sequence of **12** was determined by the analysis of FABMS and CID spectra of the protonated molecular ion (m/z 917) and a prominent fragment at m/z 476. Taken in conjunction with the observed accurate mass data, the FABMS and CID spectra are rationalized in Scheme II. All the ions shown were observed in the CID spectra while those marked with an asterisk were also observed in the FABMS of **12**. The fragmentations are those normally observed in the CID spectra of peptides.[13] With the elucidation of the structure of **12**, only the positions of the dehydroalanine moieties in **1** remained to be assigned. The stereochemistry about the threonine and the masked Ala could not be assigned by the methods used.



12 R = H R' = CH_3

13 R =  R' = CH_3

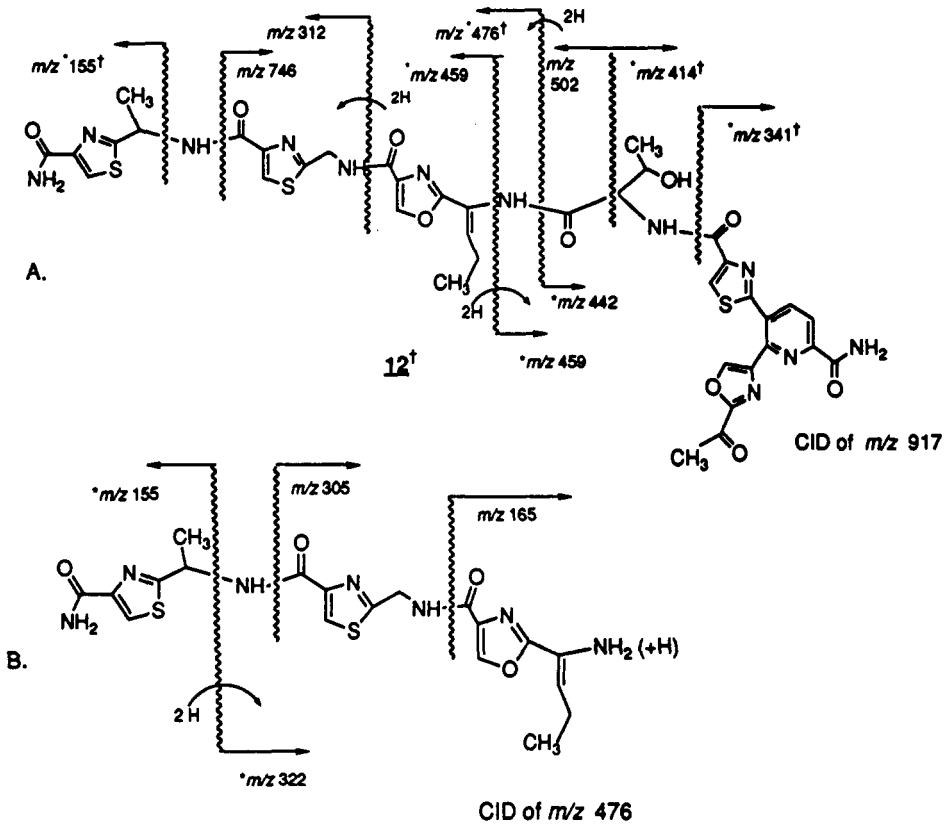
Isolated from degradation of A10255B (**1**)

17 R = H R' = H (Isolated from degradation of A10255G (**15**) and from A10255G (**16**))

^{1}H- and ^{13}C-NMR data for the major TFA degradation product were consistent with structure **12**, which was determined primarily by mass spectrometry. Homonuclear decoupling experiments allowed assignment of ^{1}H–^{1}H spin systems, although the pyridine ring protons could not be differentiated using these methods. The remaining reso-

(11) Abe, H.; Takaishi, T.; Okuda, T. *Tetrahedron Lett.* **1978**, 2791.
(12) Liesch, J. M.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1977**, *99*, 1645.
(13) Roepstrorff, P.; Fohlmann, J. *Biomed. Mass. Spectrom* **1984**, *11*, 601.
(14) The authors are indebted to the Shionogi Co., Ltd. for a generous gift of thioxamycin.
(15) Depaire, H.; Thomas, J. P.; Brun, A.; Lukacs, G. *Tetrahedron Lett.* **1977**, 139.
(16) Liesch, J. M.; McMillan, J. A.; Pandey, R. C.; Paul, I. C.; Rinehart, K. L., Jr.; Reusser, F. *J. Am. Chem. Soc.* **1976**, *98*, 201.

Scheme II[a]



[a] Key: †elemental composition supported by accurate mass measurement of ions in the FABMS of **12**.

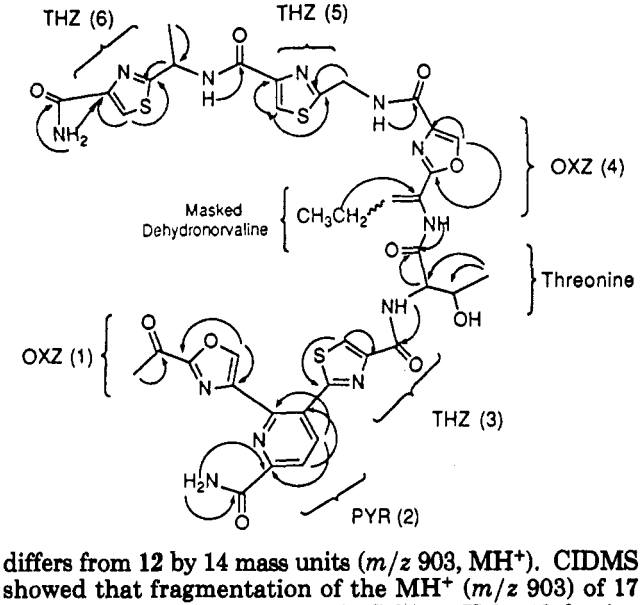
nances were all singlets and could not be specifically assigned by decoupling or NOE interactions.

The remaining ^{1}H- and most of the ^{13}C-NMR signals were assigned using heteronuclear long-range ^{1}H/^{13}C correlations obtained by the fully coupled method.[17] The observed correlations for structure **12** are shown in Scheme III, and the ^{1}H- and ^{13}C-NMR assignments are given in Table III. All of the H-5 protons in the thiazole/oxazole rings show long-range correlations to the C-2 and C-4 carbons of their respective rings, allowing specific ^{1}H and ^{13}C NMR ring assignments. In addition, the α-protons show correlations to the attached rings (C-2) for THZ-5 and THZ-6. The correlation between C-4 and a primary amide proton confirms THZ-6 as the terminal residue, and assignment of the remaining thiazole as THZ-3 can be made by default. The carbons and protons of the oxazole ring could not be assigned on the basis of the correlations observed. However, these shifts and those reported by Abe for the sulfomycinamic acid moiety in closely related antibiotic, sulfomycin I (**5**), showed exact correspondence, for the pyridine, OXZ-1, and THZ-3 subunits.[4] The carbonyl carbon atoms were assigned through correlations from nearby amide or methyl protons while the aliphatic carbons were assigned through one-bond correlations to their attached protons. The stereochemistry of the propylidene group could not be determined.

The CIDMS of the MH$^+$ of **13** (m/z 986) showed a fragment at m/z 511 (m/z 442 from fragmentation of **12** + deala), as well as a fragment at m/z 900 (MH$^+$ – deala). These data place the deala in **13** at the carboxyl group of the sulfomycinamic acid residue.

The trifluoroacetolysis of A10255G (**15**) and A10255J (**16**) produced a common degradation product, **17**, which

Scheme III



differs from **12** by 14 mass units (m/z 903, MH$^+$). CIDMS showed that fragmentation of the MH$^+$ (m/z 903) of **17** to be analogous to that shown in Scheme II for **12**, having the same sequence but possessing one less methylene unit. The only significant differences was in the sequence of fragments about the dehydronorvaline (m/z 462, 445, and 488), each being 14 mass units less than the corresponding peaks in **12**. Analysis of the CIDMS results led to the conclusion that in **17** a dehydrobutyrine residue replaces the dehydronorvaline in **12**. This conclusion in supported by ^{1}H- and ^{13}C-NMR data (Tables I and II) which show that **15** and **16** have a dehydrobutyrine (dehydro-α-aminobutyric acid) residue in place of the dehydronorvaline present in A10255B (**1**). Except for this substitution, NMR data indicate that A10255B (**1**) and

(17) Bax, A. Ed. *Two Dimensional Nuclear Magnetic Resonance in Liquids*; Reidel: London, 1986; p 52.

Table III. ^{1}H- and ^{13}C-NMR Data for A10255B Fragment 12[a]

	assignment[b]	^{13}C	^{1}H
OXZ (1)			
	2	156.62	
	4	140.56	
	5	144.10	9.30 (s)[c]
	2-C=O	185.04	
	CH_3	26.19	2.29 (s)
PYR (2)			
	2	146.20	
	3	128.81	
	4	140.92	8.38 (d)
	5	121.23	8.18 (d)
	6	150.78	
	6-C=O	164.98	
	NH_2		8.49/7.95 (bs/bs)
THZ (3)			
	2	163.77	
	4	149.41	
	5	126.77	8.58 (s)
	4-C=O	160.32	
	NH		7.99 (d)
threonine			
	CHN	58.11	4.61 (dd)
	CHO	66.99	4.24 (dq)
	CH_3	20.40	1.13 (d)
	C=O	169.21	
	NH		9.67 (s)
	OH		5.19 (bd)
dehydronorvaline			
	C=	122.21	
	CH=	134.72	6.45 (t)
	CH_2	20.66	2.21 (dq)
	CH_3	12.91	1.02 (t)
OXZ (4)			
	2	159.80	
	4	136.07	
	5	141.94	8.63 (s)
	4-C=O	160.57	
	NH		9.21 (t)
THZ (5)			
	2	170.44	
	4	148.91	
	5	124.97	8.26 (s)
	4-C=O	160.32	
	NH		9.17 (d)
	2-CH_2	40.61	4.78 (ddd)
THZ (6)			
	2	174.38	
	4	149.94	
	5	123.84	8.14 (s)
	4-C=O	162.32	
	NH_2		7.69/7.59 (bs/bs)
	2-CH	47.16	5.43 (dq)
	CH_3	20.14	1.68 (d)

[a] Solvent: DMSO-d_6. [b] Refers to designations in Scheme III; numerals refer to positions on the respective heterocyclic rings or the positions to which a particular group is attached. [c] Multiplicity.

A10255G (15) are identical in structure. A10255J (16) is identical to 15 except that it has only three deala equivalents instead of six. These data support the conclusion that A10255G (15) and A10255J (16) each have the same cyclic peptide core with different deala side chains.

Location of Deala Residues. The task of assignment of the positions of the six deala residues was addressed next. The presence of a sulfomycinamic acid group in 1 located one masked deala residue on the oxazole arm of this acid.[4] The mass spectral cleavage results for 1 and its sodium borohydride reduction product 7, shown in eq 1, require that there be a string of at least three deala units at the C-terminus of 1. The isolation of 13 as a hydrolysis product from 1 places a deala residue at the sulfomycinamic acid carboxyl group.

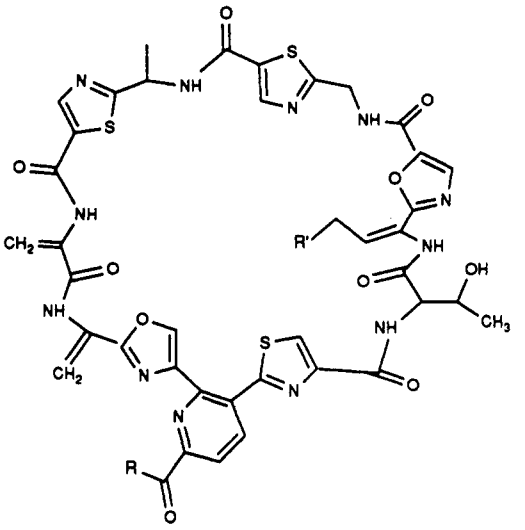
On the basis of HRFABMS and CIDMS comparisons to 1, the replacement of dehydrobutyrine for dehydronorvaline is the only difference between 15 and 1. HRFABMS and CIDMS data revealed that 16 had three fewer deala units than 15, no sequential loss of deala residues, and the presence of a terminal carboxamide group. One of the deala α-carbons in 16 shows strong long-range correlations to a carboxamide as well as to its adjacent methylene group; these data would be possible only if this deala residue is external to the peptide core. Mass spectral evidence for cleavage of a single external deala group was entirely lacking, however. Since 15 and 16 have the same cyclic peptide core and differ only in the deala side chain, the maximum number of deala equivalents within the A10255 peptide cores must be two. Even though cleavage of only three deala units is seen in the mass spectrum of 1, the minimum number of deala residues in the side-chain string for 1 and 15 must be four.

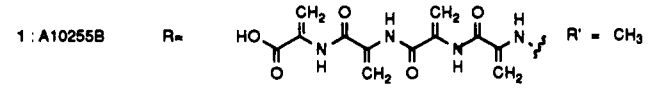
The length of the dehydroalanine string in 1 was confirmed by chemical means. Hydrolysis of 1 with aqueous base gave a new product (14) whose mass spectrum revealed the presence of a carboxyl group (m/z 952 [M – OH], m/z 925 [M – CO_2], and m/z 908 [M – OH – CO_2]). The elemental composition of 14 indicated that this product resulted from the simple loss of the elements of a deala tetrapeptide ($C_{12}H_{12}N_4O_4$) from 1 and the formation of a carboxyl group at its point of attachment to the peptide core. This provided independent evidence that the string of dehydroalanines consisted of four rather than three residues and accounted for four of the five deala residues present in 1.

The relationship between degradation products 12 and 14 established the location of the remaining deala. Since 14 differed from 1 mainly by the absence of the tetrapeptide string and was formed without hydrolytic cleavage of any additional bonds, it must be the unmodified cyclic peptide core of 1. The composition of 14 is the sum of the elements of product 12 plus a deala group which is incorporated into the core ring. Product 12, formed by TFA degradation of 1, contains no deala moieties and has a methyl ketone group at the oxazole arm of the sulfomycinamic acid unit which also possesses carboxamides at the thiazoyl as well as the pyridoyl terminii. Each of these are potential cyclization points. It has been shown that TFA hydrolysis of dehyroalanyl peptides will convert the N-acyl group attached to the amino group of a deala residue into a carboxamide group.[9] The two carboxamide groups in 12 likely originate in this manner with each having been linked to the N-terminus of a deala. This cyclization would then involve acylation of the amino group of the core deala by the thiazole carboxyl group (THZ-6) in 12 while the deala carboxyl group acylates an enamino group formed at the acetyl group on the sulfomycinamic acid moiety. This mode of cyclization satisfies composition requirements in 14 and is exactly analogous to the structures of the closely related thiopeptides 4[3,4] and 5.[4] These data are consistent with the assignment of 14 as the peptide core of A10255B (1).

The point of attachment of the tetrapeptide string was deduced from structure 13 to be at the pyridoyl carboxyl group on the sulfomycinamic acid residue. Therefore, structure 1 is assigned to A10255B. This structure resembles thioxamycin, possessing a dehydronorvaline residue in place of a *S*-methyl-L-cysteine.[5] The ^{1}H- and ^{13}C-NMR assignments, shown in Tables I and II for 1, 15, and 16, were made by comparison of data for 12 and 2D long-range ^{1}H/^{13}C correlations. The assignments for the dehydroalanines (and masked dehydroalanines) were not

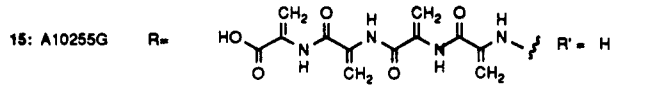
specifically determined due to the lack of ^1H NMR couplings and long-range $^1H/^{13}C$ correlations to neighbors.



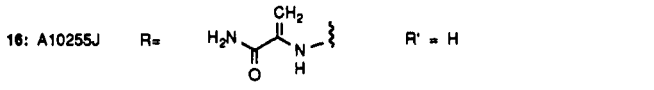


1 : A10255B R= R' = CH_3

14 : R = OH ; R' = CH_3



15: A10255G R= R' = H



16: A10255J R= R' = H

Mass Spectral Observations. Multiple units of dehydroalanine arranged in strings or chains are a common structural feature among the thiopeptides [e.g., berninamycin A (**4**),[3] sulfomycin I (**5**),[4] and, more recently, thioxamycin (**6**)].[5] However, the mass spectral fragmentation of these chains has not been studied in detail. It was tempting to conclude that the peptide string in **1** consisted of a string of three deala residues based on the CIDMS data summarized in eq 1. However, there remained a distinct possibility that structural features near the point of attachment of the deala string to the peptide core might strongly interfere with the fragmentation of the last residue in the string. Therefore, corroborating evidence was sought.

The structure of thioxamycin (**6**) was recently reported but without documentation. The published proposed structure has a tetradeala string similar to that present in **1** and **15** and thus provided an opportunity to test the generality of the mass spectral fragmentation behavior of deala chains described above.[5,14] The nature of the fragmentation of the tetradehydroalanyl peptide chain of **6** was studied using the unimolecular dissociation spectrum of its FABMS molecular ion (MH^+ m/z 1265). The molecular ion of **6** sequentially lost only three of the four deala residues present in its tetrapeptide string as high intensity fragments: m/z 1178 [MH^+ −87, (loss of one deala + OH)], m/z 1109 [MH^+ − (two deala + OH)], and m/z 1040 (MH^+ − (three deala + OH). The loss of the fourth deala residue by fragmentation occurred only with great difficulty and was observed as a peak of very low intensity at m/z 971 (MH^+ − (four deala + OH)) by accumulation of data from several scans. On the basis of the mass spectral fragmentation of **1** and **15** it can be predicted that **6** has a string of four deala groups as the undocumented structure depicts. Accumulation of data for the unimolecular dissociation of the molecular ion of **1** revealed an analogous low-intensity peak at m/z 951 (MH^+ − (four deala + OH)) in addition to the high-intensity fragmentations of its tetrapeptide described above. In the limited number of cases studied here, there is strong evidence that the intensity of the fragmentation of the deala residue closest to the point of attachment is severely inhibited by the structural environment about the sulfomycinamic acid residue. This observation may provide a valuable tool for the study of such groups which appear to be widely distributed to the thiopeptide antibiotic class.

Concluding Remarks. The structure of A10255B shows several similarities to berninamycin A, sulfomycin I, and thioxamycin. Each of these thiopeptides has a large monocyclic peptide core to which is attached a deala string of variable length. The cyclic peptides are similar in size and have a high degree of structural homology. Whereas berninamycin A and sulfomycin I are neutral compounds (C-terminal amide), A10255B and thioxamycin are acidic (C-terminal COOH).

Experimental Section

General Remarks. NMR spectra were obtained with Bruker AM 500 and Bruker WM-270 spectrometers. FAB mass spectra were obtained using a V6 ZAB-2SE mass spectrometer, and CID spectra were obtained using either the SAB-2SE or a V6 ZAB-3F mass spectrometer. Samples were dispersed in a 5:1 mixture of dithiothreitol/dithioerythritol. TLC was carried out using E. Merck plates of Silica gel 60 with a fluorescent indicator (F_{254}). Amino acid analyses were done on a Beckman 120C amino acid analyzer equipped with a Bio-Cal autosample applicator and programmer and single Durrum BC-1A resin.

Isolation of the A10255 Antibiotic Complex. A10255 was isolated from the fermentation of *Streptomyces gardneri*, NRRL 15537, or NRRL 18260 under submerged aerobic conditions using conditions outlined by Michel et al.[2] The mycelial cake was obtained by filtration and extracted twice with acetone/H_2O (8:2). The extract was concentrated to give a precipitate which was isolated by centrifugation and dried under reduced pressure. The solid was analyzed by HPLC using an IBM 5UM C_{18} column (4.5 × 50 mm) run at a flow rate of 1.5 mL/min (linear gradient from 0.05 M NH_4OAc (pH 5.5)/CH_3CN (80:20) (solvent A) to 0.05 M NH_4OAc (pH 5.5)/CH_3CN (60:40) (solvent B), gradient from 25% to 75% B. The crude product contained 60–80% of a mixture of several related components including A10255B (retention time 7.9 min, 7.1% present in complex), -G (6.92 min, 84%), -H (9.0 min, 3.2%), and -J (8.5 min, 3.3%). Other components were present at levels below 1% (see below); among these are A10255C (9.7 min) and -E (9.1 min).

Purification of the A10255 Components. The A10255 components were isolated and purified by preparative reversed-phase chromatography using a LP-1 C_{18} column cartridge (PREP 500, Waters Associates) run at a flow rate of 40 mL/min and a linear gradient from solvent A [$H_2O/CH_3CN/THF/HOAc$ (70:30:5:0.5)] to solvent B [$H_2O/CH_3CN/THF/HOAc$ (65:35:5:0.5)] collecting 250 mL. Every other fraction was analyzed by HPLC, and combinations were made based on enrichment. The first 15 fractions were discarded. The remaining fractions were combined, and equivalent combinations from several chromatographic separations were pooled on the basis of retention time and repurified by repeated HPLC under the same conditions. The results of the repurification of the above combination are as follows.

A10255J (**16**): HRMS m/z 1023 (MH^+) calcd for $C_{43}H_{38}N_{14}O_{11}S_3$ 1023.208, found 1023.205; UV (EtOH) 246 (62200); IR (KBr) 1665, 1596, 1523 cm^{-1}; pK_a (66% DMF) 13.1.

A10255G (**15**): HRMS m/z 1231 (MH^+) calcd for $C_{52}H_{47}N_{16}O_{15}S_3$ 1231.2569, found 1231.2533; UV (EtOH) 247 nm (75200); IR (KBr) 1662, 1538, 1515, 1491 cm^{-1}; pK_a (66% DMF) 5.3, 11.5.

A10255B (**1**): HRMS m/z 1245 (MH^+) calcd for $C_{53}H_{48}N_{16}O_{15}S_3$ 1245.2726, found 1245.2672; UV (EtOH) 247 nm (78700); IR (KBr) 1539, 1664 cm^{-1} (several amides); pK_a (66% DMF) 5.0, 11.3, ~13.5.

Components A10255A, -C, -E, and -H did not undergo further structure elucidation due to the limited quantities of pure materials isolated for this purpose.

$NaBH_4$ Reduction Product 7. A solution of 1 (1.25 g, 1.0 mmol) dissolved in THF/EtOH (9:1) (50 mL) was treated with $NaBH_4$ (500 mg, 13.5 mmol) and stirred for 25 h. The reaction mixture was adjusted to pH 3.0 (1 N HCl) and stirred for 15 h at room temperature. The reaction mixture was taken to near dryness under reduced pressure. The residual solids were suspended in water and extracted three times with $CHCl_3$ and the organic extracts dried over Na_2SO_4 to give a tan solid (18 mg). Excess $CHCl_3$ in the aqueous layer was removed under reduced pressure, and concentration to half volume gave a tan precipitate (915 mg). This material was fractionated (250-mL fractions) using silica gel chromatography (Waters Prep-500, gradient solvent system: 100% CH_3CN) → 30% H_2O/CH_3CN run at 250 mL/min). Combination of fractions was directed by TLC (silica gel plates using a $CHCl_3$–MeOH (3:1) solvent system). The major product had a R_f = ~0.4 with other products having lower R_f values. After the first 10 fractions were discarded, combinations of like fractions were concentrated to near dryness and acidified to pH 2 (1 N HCl) to give a granular precipitate which was washed with water and isolated by filtration. Three major combinations were made: A, fractions 11–13 (76 mg); B, fractions 14–15 (55 mg); and C, fractions 16–32 (315 mg). A and B were repurified by preparative TLC (silica gel plates, 20 × 20 × 2 mm; $CHCl_3$/MeOH (6:1)) to give two broad bands: I (R_f = 0.8, 8 mg) and II (R_f = ~0.25, 13 mg). Each band was isolated from the silica gel by prolonged extraction with 72% methanolic $CHCl_3$, desalted by treatment with 0.1 N HCl for 30 min, filtered, and washed with H_2O. Band II gave product 7 which had the elemental composition of 1 and 12 additional hydrogens and had the following properties: HPLC retention time 92 s (0.5 N NH_4OAc in 38% CH_3CN, RCM Nova C_{18} column (4 × 300 mm) using a flow rate at 2 mL/min); amino acid analyses (Amino acid (no. of residues detected)): Thr (1.0), Ala (5.34), Norval (0.86); FAB-MS (MH^+) m/z 1257 calcd for $C_{53}H_{61}N_{16}O_{15}S_3$ 1257.3664, found 1257.3630; CIDMS of (MH^+) showed the following fragmentations for which the following masses, correct to the nearest integer, were determined: m/z 1170 (1257 - 87), 1099 (1170 - 71), 1028 (1099 - 71); ^{1}H-NMR (DMSO) δ 5.69 (s, H), 6.26 ppm (s, H), deala protons.

Methanolysis of A10255B. A solution of A10255B (300 mg, 0.24 mmol) in 50 mL of 3 N methanolic HCl was refluxed for 3 h under an inert atmosphere. The solvent was removed under reduced pressure, CH_2Cl_2 (100 mL) and H_2O (50 mL) were added and stirred, and each layer was isolated. The aqueous layer was extracted twice with CH_2Cl_2 (50-mL portions). After the residue was dried (Na_2SO_4), the combined organic extracts were evaporated under reduced pressure to give 58 mg of a gummy substance. The aqueous layer was lyophilized to give 260 mg of red-orange solid.

The isolated organic extract was purified by double elution on a preparative TLC using petroleum ether B/EtOAc (4:1) as the first solvent and EtOAc as the second after drying the plate. Six distinct bands were isolated by extraction from the absorbent with MeOH/EtOAc/$CHCl_3$ (1:1:4). The three fastest moving bands were essentially homogeneous by analytical TLC (EtOAc): (*A*) moved with the solvent front (R_f = ~0.96) (unrelated nonpolar impurities); (*B*) (R_f = ~0.86, 3.6 mg, yellow crystals), and (*C*) (R_f = ~0.63, 4.3 mg, light yellow solid).

Band B, 4-carbomethoxy-2-propionyloxazole (8): FABMS (MH^+) m/z 198, calcd for $C_9H_{12}NO_4$ 198.0766, found 198.0762; ^{1}H-NMR ($CDCl_3$) δ 1.02 (t, J = 9 Hz, CH_3), 1.79 (m, J = 9 Hz, CH_2), 3.14 (t, J = 9 Hz, CH_2), 3.96 (s, OCH_3), 8.37 (s, aryl H).

Band C (9): mp 156–160 °C (lit. mp 160.5–161.0 °C);[10] ^{1}H-NMR ($CDCl_3$) δ 2.46 (s, acetyl), 3.97 (s, CH_3O), 4.04 (s, CH_3O), 8.21 (s, 2 H, aryl), 8.36 ppm (s, 1 H, aryl), 8.39 (s, 1 H, aryl); FABMS (MH^+) m/z 388, calcd for $C_{17}H_{14}N_3O_6S$ 388.0603, found 388.0603. These data are in complete agreement with the literature values for dimethyl sulfomycinamate, isolated from 5 by Abe.[10]

The aqueous layer was lyophilized and acetylated with Ac_2O–pyridine (1:2, 15 mL) at room temperature for 18 h. The reaction mixture was taken to dryness under reduced pressure, resolubilized with a small amount of MeOH, and concentrated (repeated three times) to give 328 mg of a gum which was chromatographed over silica gel (100–200 mesh) on a 1.2- × 20-mm column packed in petroleum ether B taking 30-mL fractions. Elution was performed sequentially with petroleum ether B (one fraction), toluene (one fraction), 50% EtOAc/toluene (two fractions), 100% EtOAc (three fractions), CH_2Cl_2/EtOAc (1:9) (three fractions), CH_2Cl_2/EtOAc (2:8) (one fraction), CH_2Cl_2/EtOAc (1:1) (one fraction), and 100% CH_2Cl_2 (two fractions). The fractions were grouped by TLC, NMR, and MS. After trituration with petroleum ether B, Et_2O, and/or $CHCl_3$, purity of the products was assessed by TLC and HPLC. Fraction 11 (CH_2Cl_2/EtOAc (1:9)) was triturated with $CHCl_3$ to obtained 11 as crystalline solid (4 mg): mp 77–79 °C; FABMS m/z 369 (MH^+) calcd for $C_{14}H_{17}N_4O_4S_2$ 369.0691, found 369.0689; FABMS/MS (diagnostic fragmentations of m/z 369 (MH^+) peak m/z 170, 185, and 183 (see structure 11 for fragmentation pattern); ^{1}H-NMR ($CDCl_3$) δ 1.79 (d, J = 7 Hz, CH_3), 2.08 (s, –OAc), 3.97 (s, OCH_3), 4.75 (m, CH_2), 5.61 (m, J = 7 Hz, CC*H*NH), 6.35 (broad s, –N*H*CH_2–), 7.95 (d, J = 7 Hz, –(CH_3)-CHN*H*), 8.07 (s, 1 H, aryl), 8.13 ppm (s, 1 H, aryl).

Isolation of Berninamycinic Acid (10) from A10255B. A solution of A10255B (200 mg) in 6 N HCl (8 mL) was refluxed for 18 h under N_2. Concentration of the cooled solution to half volume gave a precipitate which was collected by filtration at 5 °C. This product had physical properties identical to those of berninamycinic acid (10):[16] FABMS m/z 291 (MH^+); ^{1}H-NMR ($CDCl_3$) δ 8.00 (1 H, d, J = 8.0 Hz), 8.39 (1 H, s), 8.41 (1 H, d, J = 8.0 Hz), 9.21 (1 H, s); UV (EtOH) 231 (17 200), 276 (17 900), 327 (6350), 368 (4720) nm; pK_a (66% DMF) 4.5, 7.4.

Degradation of A10255 Antibiotics with Trifluoroacetic Acid. (a) TFA Degradation of 1. A solution of A10255B (500 mg) in 15 mL of anhydrous trifluoroacetic acid (TFA) was stirred for 18 h at room temperature under N_2. The reaction mixture was taken to dryness under reduced pressure at room temperature to give a tan residue. Trituration of this product with Et_2O (3 × 50 mL) gave a solid that was insoluble in Et_2O which was dissolved in 50 mL of MeOH and filtered to remove a small amount of insoluble material (40 mg). The filtrate was concentrated to half volume, 15% water added, and the pH adjusted to 2.8 (0.1 N NaOH) to give a precipitate. After the suspension was concentrated to $^3/_4$ volume and cooled a solid (408 mg) was collected and vacuum dried. This material was purified in 30–60-mg loads by preparative HPLC (Prep 600, Waters Associates, RP-C_{18} silica gel column) using a gradient of 1:8:1 to 1:2:1 CH_3CN/H_2O/MeOH over a 30-min period at a flow rate of 10 mL in four separate runs. Fractions from each run were analyzed and grouped by TLC (silica gel, $CHCl_3$/MeOH (3:1)). Two major components were isolated: A, HPLC retention time 135 s, and B, HPLC retention time 120 s (RCM Nova C_{18} column (4 × 300 mm) developed at 2 mL/min with 0.5% $NH_4OAc/CH_3CN/H_2O$ (38:62). Like fractions from each run were dissolved in $CHCl_3$/MeOH (9:1), taken to dryness, and treated with H_2O to give a white powder upon drying. The A component gave 53 mg of white powder 12: FABMS m/z 917 (MH^+) calcd for $C_{38}H_{37}N_{12}O_{10}S_3$ 917.1918, found 917.1894; fragment ions m/z 476, calcd for $C_{19}H_{22}N_7O_4S_3$ 476.1175, found 476.1140; m/z 414, calcd for $C_{18}H_{16}N_5O_5S$ 414.0872, found 414.0876; m/z 341, calcd for $C_{15}H_9N_4O_4S$ 341.0345, found 341.0369; m/z 155 calcd for $C_6H_7N_2OS$ 155.0279, found 155.0261; NMR (DMSO) (see Table III). B gave 16 mg of solids. 13: FABMS MH^+ m/z 986, calcd for $C_{41}H_{40}N_{13}O_{11}S_3$ 986.2122, found 986.2157; MS/MS on MH^+ m/z 968 (986 - H_2O) m/z 900 (986 - deala), m/z 511, m/z 476; ^{1}H-NMR ($CDCl_3$) δ 6.18, 5.70 (s, deala=CH_2).

(b) TFA Degradation of 15. The identical trifluoroacetolysis conditions purification procedures described (see a) were applied to 15 (400 mg) to give 78 mg of a degradation product 17 (t_R 648 s, isocratic using CH_3CN/H_2O (25:75) containing 0.5% NH_4OAc on μC_{18}-Bondapak column (4 × 300 mm, steel, Waters Assoc.) at 2 mL/s): FABMS m/z 903 (MH^+) calcd for $C_{37}H_{35}N_{12}O_{10}S_3$ 903.1761, found 903.1761; fragmentations m/z 488, 462, 445, 442, 414, 341, 308, 155; ^{1}H-NMR (DMSO) δ 1.76 (d, J = 7.2 Hz, CH_3), 6.50 (q, J = 7.2 Hz, –CH=).

(c) TFA Degradation of 16. The identical reaction and purification conditions used in part a of this experiment were employed in the degradation of 16. The product obtained was identical to 17.

Base Hydrolysis of 1. A solution of 1 (200 mg, 0.16 mmol) in 10 mL of water was adjusted to pH 11 with 1 N NaOH was

stirred at room temperature for 18 h while this pH was maintained. A few drops of acetone were added at midreaction to inhibit foaming. The reaction was acidified to pH 2 and the resulting precipitate isolated by filtration, carefully washed with H_2O, and dried under reduced pressure to give a tan powder 14 (240 mg): FABMS MH^+ m/z 969 calcd for $C_{41}H_{37}N_{12}O_{11}S_3$ 969.1867, found 969.1890; m/z 952 [M - OH], m/z 925 [M - CO_2], and m/z 908 [M - OH - CO_2]; t_R 108 s, using a RCM Nova C_{18} column (4 × 300 mm) developed isocratically at 2 mL/min with CH_3CN/H_2O, 0.5% aqueous NH_4OAc (38:62). This product was labile and resisted further purification and characterization.

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A Convergent Approach to the Dihydrotachysterol Diene System. Application to the Synthesis of Dihydrotachysterol$_2$ (DHT$_2$), 25-Hydroxydihydrotachysterol$_2$ (25-OH-DHT$_2$), 10(*R*),19-Dihydro-(5*E*)-3-epivitamin D$_2$, and 25-Hydroxy-10(*R*),19-dihydro-(5*E*)-3-epivitamin D$_2$[†]

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Total synthesis of A-ring fragments of 10(*S*),19-dihydrovitamins D and 10(*R*),19-dihydro-(5*E*)-epivitamins D from (+)-(*S*)-carvone and (-)-(*R*)-carvone is described. These fragments were used for convergent synthesis of dihydrotachysterol$_2$ (DHT$_2$), 25-hydroxydihydrotachysterol$_2$, 10(*R*),19-dihydro-(5*E*)-3-epivitamin D$_2$, and 25-hydroxy-10(*R*),19-dihydro-(5*E*)-3-epivitamin D$_2$.

Introduction

Since the discovery that 1α,25-dihydroxyvitamin D$_3$ (**1**, Figure 1) modulates cell differentation and inhibits cell proliferation,[1] much effort has been put into the development of new vitamin D analogues of potential clinical interest.[2] The dihydrotachysterols **2a** and **2b** (Figure 1) have for a long time been used clinically as analogues of **1**[3,4] in relation of the role of the vitamin D in calcium metabolism. While their interest in this respect has declined due to the availability of 1α-hydroxylated vitamin D analogues,[5] their potential as antiproliferative drugs or as differentiation inducers remains. In spite of this, neither **2a** nor **2b**, nor their side-chain analogues, have been tested in these capacities.

We have previously reported a method for the preparation of dihydrotachysterols that is based on the regio- and stereoselective reduction of the 10,19-double bond of the corresponding 5,6-*trans*-vitamin D precursor (Scheme I).[3,6] However, this approach is inconvenient for pharmacological screening of numerous dihydrotachysterols and their hydroxylated or modified side-chain analogues, since it requires the individual preparation of each 5,6-*trans*-vitamin D precursor. We have therefore developed a strategy allowing the convergent assembly of a variety of side-chain analogues. We envisaged the diene system of dihydrotachysterol$_2$ (**2b**) and related derivatives as arising from a Wittig-Horner coupling between the α-anion of the unknown phosphine oxide **4** (contributing ring A) and the appropriate Grundmann ketone **5** (contributing the upper fragment).[7] We describe here the total synthesis of **4** and its enantiomer **6** and their use in the convergent synthesis of dihydrotachysterol$_2$ (DHT$_2$, **2b**), 25-hydroxydihydrotachysterol$_2$ (25-OH-DHT$_2$, **2c**), 10(*R*),19-dihydro-(5*E*)-3-epivitamin D$_2$ (**2d**), and 25-hydroxy-10(*R*),19-dihydro-(5*E*)-3-epivitamin D$_2$ (**2e**).

Synthesis of the Phosphine Oxide 4 and Its Enantiomer 6. Phosphine oxide **4** was synthesized from com-

[†] This work was taken in part from the Ph.D. thesis of Miguel A. Maestro (Universidad de Santiago, December 1989). This work was presented in part as a communication at the Eighth Workshop on Vitamin D, Paris, France, July 1991. See: Mouriño, A.; Granja, J.; Mascareñas, J. L.; Sarandeses, L.; Torneiro, M.; Maestro, M.; Fall, Y.; Castedo, L. Vitamin D - Gene Regulation, Structure-Function Analysis and Clinical Application, Norman, A. W., Bouillon, R., Thomasset, M., Eds.; Walter de Gruyter: New York-Berlin, 1991; p 199.

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