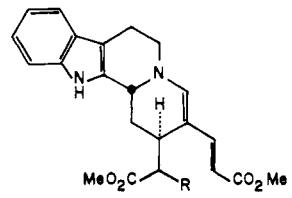
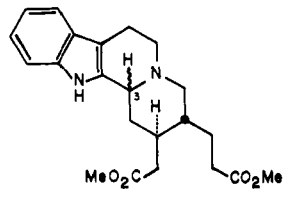


a, R = CO_2Me
b, R = H

3

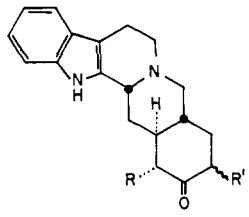


a, H(3β)
b, H(3α)

4

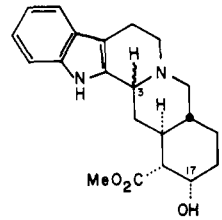
NMR ($CDCl_3$) δ 3.58, 3.67 (each s, 3), 3.91 (br s, 1), 6.9–7.5 (m, 4)).

Treatment of **4a** with sodium hydride in tetrahydrofuran (50 °C, 1.5 h) gave (44 and 36%, respectively) keto esters **5a**[2] (mp 227–228.5 °C; IR ($CHCl_3$) 3460, 1735, 1710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.87 (s, 3), 4.53 (br s, 1), 6.9–7.5 (m, 4)) and **5b**[2] (mp 223–225 °C; IR ($CHCl_3$) 3465, 1720, 1655, 1615



a, R = CO_2Me, R' = H
b, R = H, R' = CO_2Me
c, R = R' = H

5



a, H(3β)
b, H(3α)

6

cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.67 (s, 3), 4.60 (br s, 1), 6.9–7.6 (m, 4)). Alkaline hydrolysis and acid-induced decarboxylation of the latter afforded (±)-pseudoyohimbone (**5c**), mp 247–250 °C (lit.[1] mp 249–251 °C) (spectra identical with those of authentic sample), confirming the stereochemistry of all precursors. Hydrogenation of **5a** (platinum, 1:1 methanol–acetic acid, 1 drop of 36% hydrochloric acid, atmospheric pressure, room temperature, 48 h) yielded (72%) (±)-pseudoyohimbine (**6a**),[2,4,5] mp 249–251 °C dec (lit. mp[4] 252–256 °C, charring at 250 °C; mp[5] 248–251 °C) (spectra identical with those of an authentic specimen).

Hydrolysis of diester **4a** in refluxing 2:1 18% hydrochloric–acetic acids (24 h), followed by esterification with methanolic hydrogen chloride, led to the recovery (27%) of starting ester and the formation (41%) of isomer **4b:** mp 153–155 °C (lit.[6] mp 152–154 °C); IR (KBr) 3375, 1735, 1718 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.67, 3.71 (each s, 3), 7.0–7.8 (m, 4). In view of the previous conversion of the latter into (+)-yohimbine (**6b**)[6] and (−)-β-yohimbine (17-*iso*-**6b**),[6] this constitutes a formal total synthesis of these alkaloids also.[5]

Acknowledgment. The authors are indebted to the U.S. Public Health Service for support of this work.

References and Notes

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(8) NATO fellowship holder during 1975–1976.

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Structure of Mildiomycin, a New Antifungal Nucleoside Antibiotic

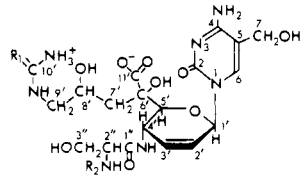
Sir:

A new nucleoside antibiotic, mildiomycin, was isolated from the culture filtrate of *Streptoverticillium rimofaciens* B-98891 in our laboratories.[1] It shows strong activity against powdery mildews on various plants[1a] and remarkably low toxicity in mammals and fishes.[1b] This paper deals with the structural elucidation of mildiomycin carried out on the basis of chemical degradations and spectral evidence as shown in Chart I.

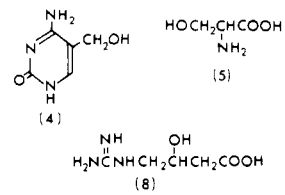
Mildiomycin (**1**)[1b] is a water-soluble, basic antibiotic: $C_{19}H_{30}N_8O_9 \cdot H_2O$; mp >300 °C dec; $[\alpha]^{23}_D$ +100°;[2] pK_a' = 2.8 (-COO^-), 4.2 (3-NH^+=), 7.2 (2″-NH_3^+), and >12 (guanidine); ν 1650 (-CONH-) and 1000–1150 (-C-O-) cm^{-1};[3] λ (pH 7) 271 nm (ε 8720) and λ (0.1 N HCl) 280 nm (ε 13 100); positive with Sakaguchi, Greig–Leaback and ninhydrin reactions. Because **1** is noncrystallizable, hygroscopic and nonvolatile, determination of the molecular formula of **1** was based on two crystalline derivatives, 2″-*N*-monobenzoate **2** ($C_{19}H_{30}N_8O_9 \cdot C_7H_4O \cdot 2H_2O$ (benzoyl chloride/5% $NaHCO_3$), mp >300 °C, $[\alpha]^{27}_D$ +92.5° ($AcOH-H_2O$ (2:8)) and 2′,3′-dihydromildiomycin (**3**, $C_{19}H_{32}N_8O_9 \cdot H_2O$ (PtO_2/water), mp >300 °C, $[\alpha]^{22}_D$ ±0°). The ^{13}C NMR spectra of **1** and **3** also support the molecular formula as shown in Table I.

On acidic hydrolysis (2 N HCl, reflux, 2 h), **1** gave 5-hydroxymethylcytosine (**4**) and L-serine (**5**), which were identified with the authentic samples. The ^{13}C NMR signals of **1**

Chart I



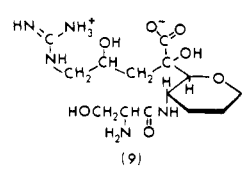
(1): R_1=NH, R_2=H (Mildiomycin)
(2): R_1=NH, R_2=Bz
(3): 2′,3′-Dihydro derivative of (1)
(6): R_1=O, R_2=H, Not ionized
(7): Formic acid salt of (1)



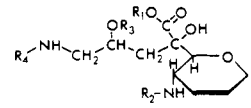
(4)

(5)

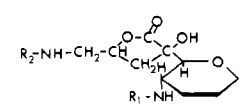
(8)



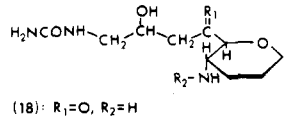
(9)



(13): R_{1-3}=H, R_4=C(=NH)NH_2·HCl
(14): R_{1-3}=H, R_4=C(=NH)NH_2
(15): R_{1-3}=H, R_4=$CONH_2$
(16): R_1=H, R_{2-3}=Ac, R_4=CONHAc
(17): R_1=CH_3, R_{2-3}=Ac, R_4=CONHAc



(10): R_1=COCH(NHBz)CH_2OH, R_2=$CONH_2$
(11): R_1=COCH(NHBz)CH_2OAc, R_2=CONHAc
(12): R_1=H·HCl, R_2=C(=NH)NH_2·HCl



(18): R_1=O, R_2=H
(19): R_1=H,OH, R_2=Ac

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which correspond to those of **4** and **5** have been assigned without any ambiguity.

The presence of a guanidyl group in **1** was established as follows. **1** was hydrolyzed (0.2 N NaOH, reflux, 2 h) to give ammonia and an ureido compound (**6**, $[\alpha]^{22}_D$ +83.0°, negative Sakaguchi and positive *p*-dimethylaminobenzaldehyde reactions). On an alternate hydrolysis (saturated $Ba(OH)_2$, reflux, 2 h), **1** afforded urea (mp 134 °C), **4**, **5**, **6**, and ammonia. A singlet signal at 158.0 ppm in the ^{13}C NMR spectrum of **1** can be reasonably assigned to the guanidyl carbon.

The aliphatic moiety was unveiled by proton spin-decoupling studies of mildiomycin formic acid salt (**7**, $[\alpha]^{22}_D$ +86.7°). On irradiation of a methine proton at 3.92 ppm (m, $H_{8'}$), two methylene signals at 1.95 (dq, $H_{7'}$) and 3.35 ppm (dq, $H_{9'}$) were collapsed into two sets of AB quartet signals; only the 8′-proton signal was decoupled when each of the methylene protons was irradiated. In the ^{13}C NMR spectrum of **1**, the signals at 48.1 and 39.2 ppm should be assigned to the $C_{9'}$ and $C_{7'}$ methylenes. These spectral data evidence that **1** has the following moiety: $>NCH_2CHCH_2C<$. Furthermore, this partial structure was elucidated from a decomposition product. **1** was subjected to periodate oxidation (6% HIO_4–2 N HCl, reflux, 2 h) to afford optically active γ-guanidino-β-hydroxybutyric acid (**8**, mp 250 °C dec, $[\alpha]^{27}_D$ −19.5°(AcOH–0.05 N HCl–MeOH (3:7:10)) which was identified with an authentic sample of the racemic compound synthesized.[4] The chemical shifts for the $C_{7'}$ to $C_{10'}$ signals in the ^{13}C NMR spectrum of **1** also receive support from the corresponding signals of **8**.

The presence of pyran-3-ene moiety in **1** was suggested by ^1H and ^{13}C NMR spectra of **1**, **3** and **7**. In the ^1H NMR spectrum of **1** an anomeric and two olefinic proton signals were observed at 6.43 (s like, $H_{1'}$), 6.05 (d like, J = 10 Hz (cis coupling), $H_{2'}$), and 5.88 ppm (d like, J = 10 Hz, $H_{3'}$), while, in the ^1H NMR spectrum of **3**, the anomeric proton shifted to 5.66 (q, J = 10 and 2 Hz, $H_{1'}$) and newly occurring methylene signals were observed at 1.8 and 1.9–2.2 ppm (2 H × 2, m, $H_{2'}$ and $H_{3'}$). The presence of two olefinic carbons at $C_{2'}$ and $C_{3'}$ was also supported by the ^{13}C NMR spectra of **1** and **3**. When the olefinic proton at 5.88 ppm ($H_{3'}$) in **7** was irradiated, the methine proton at 4.95 ppm (d like, $H_{4'}$) was sharpened. On irradiation of this methine proton, the doublet signal at 4.24 ppm (J = 10 Hz, $H_{5'}$) collapsed into a singlet, while irradiation of the 5′ proton led to collapse of the 4′ proton into a singlet-like signal. The presence of pyran-3-ene moiety is further supported by ^1H and ^{13}C NMR data in the sugar moiety of blasticidin S.[5,6]

Valuable information came from a compound (**9**, $C_{14}H_{27}N_5O_7$, $[\alpha]^{23}_D$ −21.8°, pK_a' = 3.45 (−COO^-), 7.2 (2″-NH_3^+), and >12 (guanidine), ν 1650 cm^{-1} (-CONH-)) which was obtained along with **3** and **4** by hydrogenation of **1** (PtO_2/water). In the ^1H NMR spectrum of **9**, the signals indicated that **9** lacked the pyrimidine skeleton, that the anomeric proton was lost, and that a methylene signal had newly appeared at 3.85 ppm (m, $H_{1'}$), which corresponded to the signal at 3.95 ppm (m) in the hydrogenolysis product of blasticidin S.[5] The chemical shifts and off-resonance data of pyran ring of **9** in ^{13}C NMR spectrum were in good accord with those of the product.[6] These clearly demonstrated that the pyrimidine moiety should be bound to the anomeric carbon in **1**.

Alkaline hydrolysis of **9** (0.2 N NaOH, reflux, 2 h) gave a ureido compound which was benzoylated to yield 2″-*N*-benzoate **10** ($[\alpha]^{23}_D$ +22.8°, λ 228 nm (ε 11 600), ν 1770 cm^{-1} (lactone)). On acetylation (Ac_2O/pyridine) **10** afforded 10′-*N*-3″-*O*-diacetate **11** ($[\alpha]^{23}_D$ +29.8°, ν 1780 (lactone), 1740 (OAc), and 1700 (NAc) cm^{-1}). In the ^1H NMR spectrum of **11** in Me_2SO-d_6 the signals were observed at 10.31 (s, AcHN $C_{10'}$), 8.50 (t, J = 5 Hz, -NH $C_{9'}$), 8.48 (d, J = 8 Hz, NH $C_{2''}$), 7.96 (d, J = 8 Hz, -NH $C_{4'}$), 4.34 (m, $H_{3''}$), 2.03 (s,

Table I. ^{13}C NMR Spectra of Mildiomycin and Related Compounds

compd	carbon number																			
	C-2	C-4	C-5	C-6	C-7	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′	C-7′	C-8′	C-9′	C-10′	C-11′	C-1″	C-2″	C-3″
1	157.9 (s)	165.9 (s)	107.9 (s)	142.2 (d)	58.4 (t)	81.0 (d)	126.8 (d)	133.8 (d)	44.1 (d)	80.8 (d)	79.5 (s)	39.2 (t)	67.9 (d)	48.1 (t)	158.1 (s)	178.7 (s)	175.1 (s)	57.1 (d)	64.5 (t)
3	157.4 (s)	165.6 (s)	107.3 (s)	141.7 (d)	58.5 (t)	83.9 (d)	30.1 (t)	30.3 (t)	45.9 (d)	83.4 (d)	80.0 (s)	39.7 (t)	68.2 (d)	48.0 (t)	158.0 (s)	178.9 (s)	174.9 (s)	57.2 (d)	64.4 (t)
9						68.5 (t)	25.2 (t)	31.6 (t)	47.0 (d)	83.4 (d)	80.0 (s)	39.9 (t)	68.2 (d)	48.0 (t)	158.0 (s)	179.4 (s)	173.6 (s)	57.0 (d)	63.9 (t)
12						69.2 (t)	24.8 (t)	28.9 (t)	47.9 (d)	79.3 (d)	78.3 (s)	37.3 (t)	77.5 (d)	45.1 (t)	157.9 (s)	177.8 (s)			
14						67.9 (t)	25.6 (t)	33.3 (t)	49.4 (d)	86.1 (d)	80.4 (s)	40.7 (t)	68.9 (d)	48.3 (t)	158.1 (s)	179.8 (s)			
18						68.6 (t)	23.8 (t)	27.5 (t)	48.1 (d)	80.9 (d)	210.9 (s)	43.8 (t)	66.9 (d)	45.8 (t)	162.2 (s)				
	5-hydroxymethyl cytosine[a] (**4**)					sugar moiety of blasticidin S[b]					γ-guanidino-β-hydroxybutyric acid (**8**)						L-serine (**5**)		
	149.6 (s)	160.0 (s)	105.7 (s)	145.9 (d)	57.3 (t)	80.6 (d)	126.9 (d)	133.7 (d)	47.5 (d)	78.5 (d)	175.6 (s)	39.6 (t)	67.4 (d)	47.2 (t)	158.2 (s)		173.1 (s)	57.2 (d)	61.0 (t)

[a] In CF_3COOD. [b] Determined by subtracting signals of blastidic acid[5,6] and cytosine from those of blasticidin S.

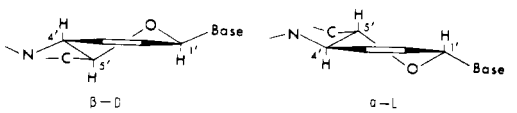
-Ac), and 1.98 ppm (s, -Ac). These data suggested that the serine moiety is bound to the 4′-amino function forming an amide bond.

Vigorous acid hydrolysis of **9** (3 N HCl, reflux, 15 h) gave a lactone dihydrochloride (**12**, $C_{11}H_{20}N_4O_4 \cdot 2HCl$, $[\alpha]^{23}_D$ ±0°) and **5**. **12** gave a monohydrochloride (**13**, $[\alpha]^{23}_D \pm 0°$) upon treatment with NH_4OH or IR-45, while a free base (**14**, $[\alpha]^{23}_D$ −7.9°, pK_a' = 2.8 (-COO^-), 8.6 (new 4′-NH_3^+), and >12 (guanidine)) was obtained by treatment with IRA-410. In the ^{13}C NMR spectrum of **14** the signals at $C_{3'}$ and $C_{5'}$ showed downfield shifts,[7] 4.4 and 6.8 ppm, when compared with those of **12**; therefore, the new primary amine should be located at 4′. The IR spectrum of **12** showed a strong absorption at 1770 cm^{-1} attributable to a five-membered lactone which disappeared in the spectra of **13** and **14**. The ^{13}C NMR signal of $C_{8'}$ shifted to 77.5 ppm in **12** from 68.9 ppm in **14**. In the ^1H NMR spectrum of **14** the signals at 3.75 (m, $H_{8'}$) and 1.95 ppm (m, $H_{7'}$) showed downfield shifts to 4.95 and 2.62 ppm (d like) in **12**, respectively. These data provided evidence for the structure of the lactone **12** as well as for the location of the carboxyl group in question.

Another ureido compound (**15**) was a key compound for establishing the location of the α-hydroxyl carboxylic acid. **14** was hydrolyzed (0.2 N NaOH, reflux, 2 h) to give ammonia and an ureido compound (**15**, mp 248 °C dec, $[\alpha]^{23}_D$ +5.9°, pK_a' = 7.75 (4′-NH_3^+) and 2.9 (-COO^-), δ 3.8 (d, $H_{5'}$) and 2.02 (m, $H_{7'}$)). On acetylation (Ac_2O/pyridine), **15** gave a triacetate (**16**, $[\alpha]^{25}_D$ +14.6°, pK_a' = 2.8 (-COO^-), ν 1700–1740 cm^{-1}, δ (Me_2SO-d_6) 1.98 (3 H, s) and 2.02 (3 H × 2, s)). On methylation (CH_2N_2/MeOH-Et_2O), **16** afforded a methyl ester (**17**, $[\alpha^{23}_D$ +14.1°, ν 1740 cm^{-1}, δ ($CDCl_3$) 3.80 (s, -$COOCH_3$).

Oxidation of **15** ($Pb(OAc)_4$/AcOH-water) yielded CO_2 and a ketone (**18**, $[\alpha]^{23}_D$ −1.2°, ν 1720 cm^{-1} (-CO-), δ 3.97 (d, $H_{5'}$) and 2.54 (m, $H_{7'}$)). In the ^{13}C NMR spectrum of **18** the signal of an isolated carbonyl group newly appeared at 210.9 ppm (s, $C_{6'}$) instead of the signal at 80.4 (s, $C_{6'}$) and 179.8 ppm (s, $C_{11'}$) in **14**. The 4′-*N*-acetate of **18** was reduced with $NaBH_4$/MeOH to give a diol (**19**, $[\alpha]^{27}_D$ +59.0°). Proton spin-decoupling studies of **19** confirmed the structure: when the 7′-methylene proton at 2.20 ppm (m) was irradiated, the methine signals at 4.25 (m, $H_{6'}$) and 4.03 ppm (m, $H_{8'}$) collapsed into a doublet (J = 7 Hz) and a double doublet, respectively. On irradiation of the methine proton at 3.78 ppm (q, $H_{5'}$) of **19**, the $H_{6'}$ methine signal at 4.25 ppm collapsed into a doublet (J = 4 Hz). From these data the presence of α-hydroxycarboxyl structure was established.

As for the absolute configuration of pyran-3-ene ring, the stereochemistry of $H_{4'}$ and $H_{5'}$ should be diaxial on the basis of the coupling constant of $J_{4',5'}$ = 10 Hz in **7**. Also the stereochemistry of $H_{1'}$ was assigned axial from $J_{1',2'}$ = 10 and 2 Hz in **3**. Thus, three bulky groups in the pyran-3-ene ring should reasonably be all equatorial. Only two sterically stable stereostructures of β-D or α-L could be permitted among all the possible isomers of pyran-3-ene as shown. Since these formula are mirror images, the Cotton effect of the CD spectrum in the B_{2u} band should be of opposite sign to each other.



β-D α-L

The CD spectra of the model and mildiomycin compounds follow: blasticidin S, $[\theta]_{270}$ −12 900; **1**, $[\theta]_{273}$ −8700; cytosinine, $[\theta]_{271}$ −9500; deseryl derivative of **1** (**20**, $[\alpha]^{24}_D$ +26.9°)), $[\theta]_{273}$ −9300; gougerotin, $[\theta]_{280}$ − 2700; **3**, $[\theta]_{285}$ −1800. The absolute configuration of blasticidin S and gougerotin has been determined as β-D.[8,9] These mildiomycin compounds showed negative Cotton effects quite similar to those of the model compounds, indicating that the pyranene ring should be β-D. The absolute configuration of **1** was thus assigned 1′*R*,4′*S*,5′*S*,2″*S*.

One of the interesting structural features of **1** is that the carboxyguanidino butyl group is bound to the unsaturated pyranoside with C-C bond. The aspects of the biosynthesis of this antibiotic provide another interesting problem—whether the quaternary carbon originates from an amino acid or sugar as a precursor.

Acknowledgments. We are grateful to Drs. E. Ohmura, K. Morita, M. Isono, and M. Yoneda in our Central Research Division for their encouragement throughout this work. We also thank the members of large-scale fermentation and physicochemical analysis sections for their support.

References and Notes

(1) (a) T. Iwasa, T. Kusaka, and K. Suetomi, *J. Antibiot.*, in press; (b) S. Harada and T. Kishi, *ibid.*, in press.
(2) Satisfactory elemental analyses were obtained for all compounds herein as containing some molecules of water of crystallization; adhesion was measured by thermogravimetric analysis. The melting points were measured by FT-5 (Mettler) at 3 °C/min. The specific rotations were also measured at the concentration of 0.5–1.0 in water unless otherwise stated.
(3) The IR spectra were measured in KBr pellet. The δ values in the ^1H and ^{13}C NMR spectra using XL-100 (Varian) were recorded in parts per million downfield from Me_4Si. All spectra herein were measured at the concentration of 20 mg/0.4 mL (^1H) and 200–300 mg/3 mL (^{13}C) in D_2O unless otherwise stated. In the ^{13}C NMR spectra dioxane was the internal standard (67.4 ppm).
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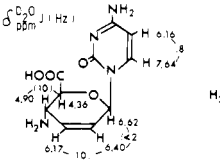
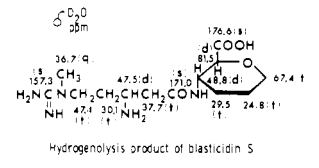
(6) Blasticidin S and its related compounds described herein were prepared by us and their spectral data were measured under the same conditions as those of our samples for comparison studies. The ^1H NMR of cytosinine and the ^{13}C NMR of hydrogenolysis product of blasticidin S follow.





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Received February 27, 1978

^{13}C-Enriched *S*-Methylmethionyl Residues as a Probe of Protein Conformation[1]

Sir:

Specific ^{13}C labeling of proteins has enhanced the usefulness of ^{13}C NMR spectroscopy as a tool for the study of these macromolecules. One highly selective method for ^{13}C enrichment of proteins which permits their observation in an essentially native form is the methyl exchange reaction at methionyl residues.[2] This method has now been applied in our laboratories to the basic pancreatic trypsin inhibitor (BPTI).[3] In the course of this work we have had occasion to make spectroscopic observations on the ^{13}C labeled protein intermediate, which possesses an enriched *S*-methylmethionyl residue at position 52 ([ε-^{13}C-SMM-52]-BPTI). Detailed NMR spectroscopic studies of *S*-methylmethionine-containing

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