ARGINOMYCIN: PRODUCTION, ISOLATION, CHARACTERIZATION AND STRUCTURE

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Arginomycin is a new nucleoside antibiotic produced by *Streptomyces arginensis*. Arginomycin, $C_{18}H_{28}N_8O_5$, which inhibits the growth of Gram-positive bacteria and fungi *in vitro*, is structurally related to blasticidin S and found to be relatively non-toxic.

Cultures of *Streptomyces arginensis* UC 8632, grown in a complex medium were found to contain an antibacterial agent which was characterized by its paper chromatographic and TLC behavior and its antibacterial spectrum. This antibiotic which exhibited activity against both Gram-positive bacteria and fungi was isolated and designated arginomycin (U-72,026). The present communication deals with the production, isolation, characterization and the chemical structure of arginomycin.

Experimental

Assay and Testing Procedures

Antibiotic production and purification was measured by a microbiological disc-plate assay procedure with *Micrococcus luteus* or *Penicillium oxalicum* as the assay organism.

Paper Chromatographic Procedures

The production of arginomycin was followed by paper chromatography using a variety of mobile phases. The Rf values of arginomycin in the following solvents were: 0.00 [1-butanol - water (84 : 16)]; 0.10 [1-butanol - water (84 : 16) containing 0.25% *p*-toluenesulfonic acid]; 0.22 [1-butanol - acetic acid - water (2 : 1 : 1)]; 0.00 [2% piperidine in 1-butanol - water (84 : 16)]; 0.70 [1-butanol - water (4 : 96)]; 0.80 [1-butanol - water (4 : 90) containing 0.25% *p*-toluenesulfonic acid]; 0.82 (0.5 M phosphate buffer, pH 7.0); 0.80 (0.075 M NH_4OH saturated with methyl isobutyl ketone, lower phase); 0.00 (benzene - methanol - water (1 : 1 : 2, upper phase); 0.10 [1-butanol - water (84 : 16) containing 2% *p*-toluenesulfonic acid]; 0.40 [methanol - 15% aqueous sodium chloride (4 : 1)].

Thin-layer Chromatographic (TLC) Procedures

TLC's of arginomycin were run on cellulose (Brinkman Cell 300) plates using pH 7.0, 0.1 M phosphate buffer as the mobile phase. Bioautography was on *M. luteus* or *P. oxalicum*-seeded trays. Hydrolytic reactions on arginomycin were followed by TLC using silica gel and chloroform - methanol - conc ammonium hydroxide (1 : 4 : 1) as the mobile phase. Spots were developed by spraying with ninhydrin or using iodine vapors. UV absorbing materials were detected by a shortwave UV lamp.

High Performance Liquid Chromatography (HPLC)

All HPLC was carried out using a C-18 reverse phase silica column on a Varian 5560 instrument equipped with an LKB-RSB photo diode array UV detector and operating in the dual pump mode. The mobile phase consisted of a 10 mM solution of hexanesulfonic acid in acetonitrile - water (80 : 20) containing 0.2% glacial acetic acid. Flow rate 1.5 ml/minute. Sample volume 10 μl.

Spectroscopic Methods

^1H NMR spectra were recorded on Bruker AM-300 and WM-500 spectrometers operating at 300 and 500 MHz, respectively. Solutions (*ca.* 0.4 ml, *ca.* 0.25 M) of the compounds in D_2O were used. ^{13}C NMR spectra were recorded on a Bruker AM-300 spectrometer operating at 75 MHz. ^1H and ^{13}C NMR chemical shifts are reported as ppm relative to tetramethylsilane. MS were obtained on a ZAB-2F high resolution (HR) mass spectrometer using a fast atom bombardment (FAB) source.

Fermentation Procedures

Shake Flask Fermentations: Seed cultures of *S. arginensis* were prepared in a medium consisting of; blackstrap molasses 5.8 g/liter, Difco peptone 10 g/liter, Difco yeast extract 4 g/liter, dextrin 4 g/liter, L-asparagine 0.2 g/liter, $CoCl_2 \cdot 6H_2O$ 1 mg/liter, pH 7.2. The cultures were incubated at 28°C for 72 hours on a rotary shaker. Fermentation medium (glucose monohydrate 20 g/liter, soybean meal 20 g/liter, brewer's yeast 2 g/liter, pH 7.2) was inoculated at a rate of 5% with the 72-hour seed culture. Fermentations were incubated at 28°C on a rotary shaker and analyzed for antibiotic production by TLC and paper chromatography and bioactivity determination. Peak titers were obtained after 72~96 hours of incubation.

Isolation of Arginomycin

Amberlite IRC-50 (H^+) Chromatography: The whole broth, 40 liters, was filtered with the aid of diatomaceous earth. The mycelial cake was washed with 4 liters of water. The wash, and clear filtrate were combined, adjusted to pH 8.5 with aqueous ammonium hydroxide and passed over a column containing 4 liters of Amberlite IRC-50 cation exchange resin (in the hydrogen form). The chromatography was followed by determination of bioactivity and bio-TLC. The spent filtrate was found bio-inactive and was discarded. The column was washed with 12 liters of water. The aqueous wash was also bio-inactive and was discarded. Arginomycin was eluted from the column with 2 N aqueous ammonium chloride. Fractions containing arginomycin were combined, the solution was adjusted to pH 8.0 with aqueous ammonium hydroxide and further processed over Amberlite XAD-4 as described below.

Amberlite XAD-4 Chromatography: The arginomycin-containing eluate from the Amberlite IRC-50 (H^+) chromatography (see above) was passed over a column containing 3.2 liters of Amberlite XAD-4. The spent was found bio-inactive and was discarded. The column was washed with 12 liters of water. The aqueous wash was also discarded. Arginomycin was eluted with methanol - water (70 : 30). Bioactive methanolic eluates were analyzed by TLC and fractions containing arginomycin only were combined, concentrated to an aqueous solution and freeze-dried to yield 4.83 g of a preparation of arginomycin which was further purified by counter-current distribution as shown below.

Counter-current Distribution: The solvent system consisted of 1-butanol - 0.1 M phosphate buffer, pH 6.5, (1 : 1). The starting material, 4.83 g of purified arginomycin obtained as described above, was dissolved in 100 ml of the lower phase of the above system. The solution was introduced in the first 10 tubes of an all-glass Craig counter-current distribution apparatus (10 ml/phase). Upper phase (100 ml) was added and the distribution was carried out for 500 transfers. The distribution was analyzed by UV, bioactivity determination and TLC. Fractions containing arginomycin were combined; the 1-butanol containing solution was then concentrated to an aqueous and freeze-dried to yield 8.54 g of a mixture of arginomycin and inorganic salt. Removal of the salt was obtained by the Amberlite XAD-4 chromatography as shown below.

Amberlite XAD-4 Chromatography for Isolation of Pure Arginomycin: The column was prepared from 1 liter of Amberlite XAD-4 in water. The freeze-dried material (*ca.* 8.5 g) obtained by counter-current distribution was dissolved in 200 ml of water. This solution was adjusted to pH 8.0 with aqueous ammonium hydroxide and added on the top of the column. The spent was collected as one fraction and, after testing for bioactivity, was discarded. The column was washed with 3 liters of water. The aqueous wash, after analysis by UV and bioactivity, was also discarded. The column was eluted with methanol - water (70 : 30), fractions containing arginomycin were combined, concentrated to an aqueous solution, and freeze-dried to yield 2.3 g of pure arginomycin. Characterization of this material, which was found homogeneous by TLC and HPLC, is described later in this

paper.

Arginomycin Hydrochloride (1a)

Arginomycin, 300 mg, was dissolved in 10 ml of 1 N methanolic hydrogen chloride. The clear solution was mixed with 100 ml of acetone and 50 ml of ether. The precipitated colorless material was isolated by filtration and dried; yield 330 mg. IR and ^{1}H NMR spectra of arginomycin hydrochloride are discussed in the characterization section.

Arginomycin Methyl Ester Hydrochloride (1b)

Arginomycin, 500 mg, was dissolved in 20 ml of 0.8 N methanolic hydrogen chloride. This solution was kept at reflux for 3 hours; it was then cooled and clarified by filtration. The clear solution was mixed with 200 ml of acetone and 50 ml of ether. Arginomycin methyl ester hydrochloride was isolated as a colorless amorphous solid by filtration; yield 520 mg. Characterization of this material is described in the discussion section of this paper.

Arginomycoic Acid (2)

Acid Hydrolysis of Arginomycin: Arginomycin, 5.15 g was dissolved in 70 ml of 3 N aqueous sulfuric acid. The solution was kept at 95°C for 46 hours. The course of the reaction was monitored by TLC [silica gel; chloroform - methanol - water - conc ammonium hydroxide (1 : 4 : 1 : 1)] and by HPLC. The reaction mixture was then diluted to a total volume of 250 ml with water and neutralized (pH 7.0) by addition of saturated barium hydroxide solution. Precipitated barium sulfate was removed by centrifugation; the supernatant was freeze-dried to give 3.13 g of a tan-colored amorphous solid.

Isolation of Cytosine and Arginomycoic Acid by Silica Gel Chromatography

The column was prepared from 400 g of silica gel packed in the mobile phase consisting of chloroform - methanol - water - conc ammonium hydroxide (1 : 4 : 1 : 1). The starting material, 1.0 g of the tan-colored material isolated as described above was dissolved in 5 ml of water and mixed with 20 g of silica gel. The mixture was dried and the dried powder was added on the top of the column which was eluted with the above mentioned mobile phase. Fractions were analyzed by TLC (UV and ninhydrin detection). Early fractions were found to contain a UV absorbing (*ca.* 269 nm) material; they were combined and concentrated to dryness to yield a material with properties (IR, UV, HPLC, FAB-MS *Anal* data) identical to those of cytosine.

Late fractions containing arginomycoic acid were combined and the solution was concentrated to dryness *in vacuo*. The residue was triturated with 20 ml of boiling ethanol. Insoluble material was separated by filtration; the filtrate was cooled and mixed with 200 ml of ether under stirring. Arginomycoic acid was isolated as amorphous colorless solid by filtration; yield 243 mg; Rf 0.5 [silica gel; chloroform - methanol - water - conc ammonium hydroxide (1 : 4 : 1 : 1)]; $[\alpha]_D^{25}$ +15° (*c* 0.56, water); molecular composition, $C_8H_{18}N_4O_2$; calcd for $(M+H)^+$ ($C_8H_{19}N_4O_2$) 203.1508; found by HRFAB-MS, 203.1509; mp *ca.* 200°C (with decomposition); IR (Nujol) cm^{-1} 3581, 3386, 3357, 2684, 2628 (sh), 1715, 1665, 1627, 1558, 1352, 1309, 1252, 1207, 1169, 1146, 1135, 1077, 1039, 1018, 968, 948, 904, 890, 878, 836, 823, 807, 735; ^{1}H NMR (D_2O) δ 1.05 (3H, d, CCH_3), 1.5~1.8 (2H, m, 4-CH_2), 2.0 (1H, m, 3-CH), 3.08 (3H, s, NCH_3), 3.40 (2H, t, 5-CH_2), 3.48 (1H, d, 2-CHN); ^{13}C NMR (D_2O) δ 15.52 (q, CCH_3), 28.71 (t, C-4), 33.49 (d, C-3), 35.54 (q, NCH_3), 48.63 (t, C-5), 59.86 (d, C-2), 156.27 (s, NCN), 179.87 (s, C-1); UV (MeOH) only end absorption.

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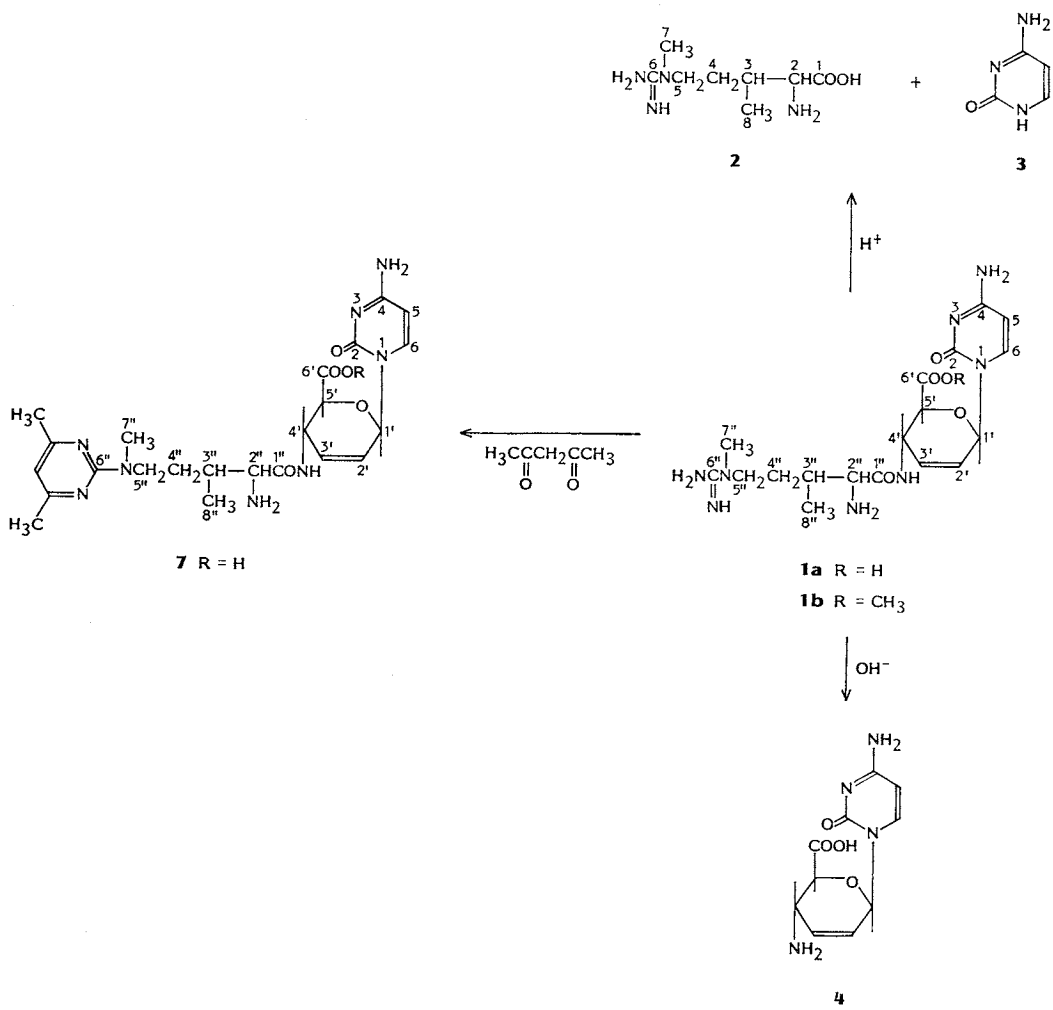
Reaction of Arginomycin with 2,4-Pentandione

The procedure described by MAEHR *et al.*[1] was followed. A mixture of 200 mg of arginomycin in 1.2 ml of water, 1.0 ml of absolute ethanol, 1.0 ml of 2,4-pentandione and 120 mg of sodium bicarbonate was kept at 90°C for 3 hours. The mixture was then concentrated to dryness, dissolved in 5 ml of 2 N acetic acid and passed over a column containing 10 ml of Dowex 50-X4 (H^+). The *N*-(4,6-dimethylpyrimidin-2-yl) derivative of arginomycin (compound **7**, Fig. 1) was obtained by eluting the column with water - pyridine (9 : 1) which was freeze-dried to yield 180 mg of a colorless material. FAB-MS gave M^+ at *m/z* 500 (calcd for $C_{23}H_{32}N_8O_5$, 500). Other ions appear at *m/z*

Fig. 1.



451, 390, 221, 204, 190, 176, 164, 150, 138, 124, 112, 96, 84 and 80. ^{13}C NMR (D_2O) δ 156.08 (s, C-2), 164.77 (s, C-4), 95.24 (d, C-5), 141.31 (d, C-6), 78.36 (d, C-1′), 124.77 (d, C-2′), 131.44 (d, C-3′), 44.99 (d, C-4′), 76.06 (d, C-5′), 168.01 (s, C-6′), 173.16 (s, C-1″), 56.91 (d, C-2″), 30.85 (d, C-3″), 27.55 (t, C-4″), 45.25 (t, C-5″), 159.27 (s, C-6″), 34.27 (q, C-7″), 13.68 (q, C-8″). The two methyl groups of the dimethylpyrimidine moiety of **7** appear as a singlet at δ 21.42. The HCC pyrimidine carbon appears at δ 107.95 (d). The two symmetrical carbons attached to the methyl groups and the nitrogens of the dimethylpyrimidine moiety appear as a singlet at δ 166.87. In the ^1H NMR spectrum (D_2O) the two methyl groups of the dimethylpyrimidine appear as a singlet at δ 2.22 (6H). The NCH_3 and CCH_3 groups of **7** appear at δ 3.0 (3H, s) and 1.05 (3H, d), respectively. UV λ_{max} nm (ε) 244 (19,000), 277 (12,000), 314 (5,000).

Results and Discussion

Isolation of Arginomycin

The production of arginomycin by *Streptomyces arginensis* was followed by bioassays vs. *Peni-*

cillium oxalicum and *Micrococcus luteus* and paper chromatography. Peak titers of arginomycin were obtained after incubation of cultures of *S. arginensis* for 72 to 96 hours.

Arginomycin exhibited the properties of an amphoteric compound. It was isolated from clear filtrates by adsorption on Amberlite IRC-50 (H^+) followed by elution with 2 N aqueous ammonium chloride. Chromatography over Amberlite XAD-4 yielded purified arginomycin free of sodium chloride. Pure arginomycin was isolated by counter-current distribution using 1-butanol - 0.1 M phosphate buffer, pH 6.5 (1 : 1) followed by desalting over Amberlite XAD-4 resin.

Characterization of Arginomycin

Arginomycin was isolated as an amorphous colorless solid soluble in water and lower alcohols and insoluble in ethyl acetate, acetone, ether and chlorinated and saturated hydrocarbon solvents. HR mass spectrometry indicated a molecular formula of $C_{18}H_{28}N_8O_5$; calcd molecular weight, 436.2183; found by HRFAB-MS, 436.2177. The FAB-MS spectrum in the positive ion mode shows a molecular ion peak at *m/z* 437 $(M+H)^+$ while the molecular ion in the negative ion mode FAB-MS spectrum appears at 435 $(M-H)^-$. Other important ions are presented in Table 1. Arginomycin melts at *ca.* 218°C and has a specific rotation of +44° (*c* 0.5, water). The UV spectra of arginomycin show λ_{max} nm (ε) at 268 (6,100) in water, 268 (6,200) in 0.1 N aqueous sodium hydroxide and 276 (8,500) in 0.1 N aqueous hydrochloric acid.

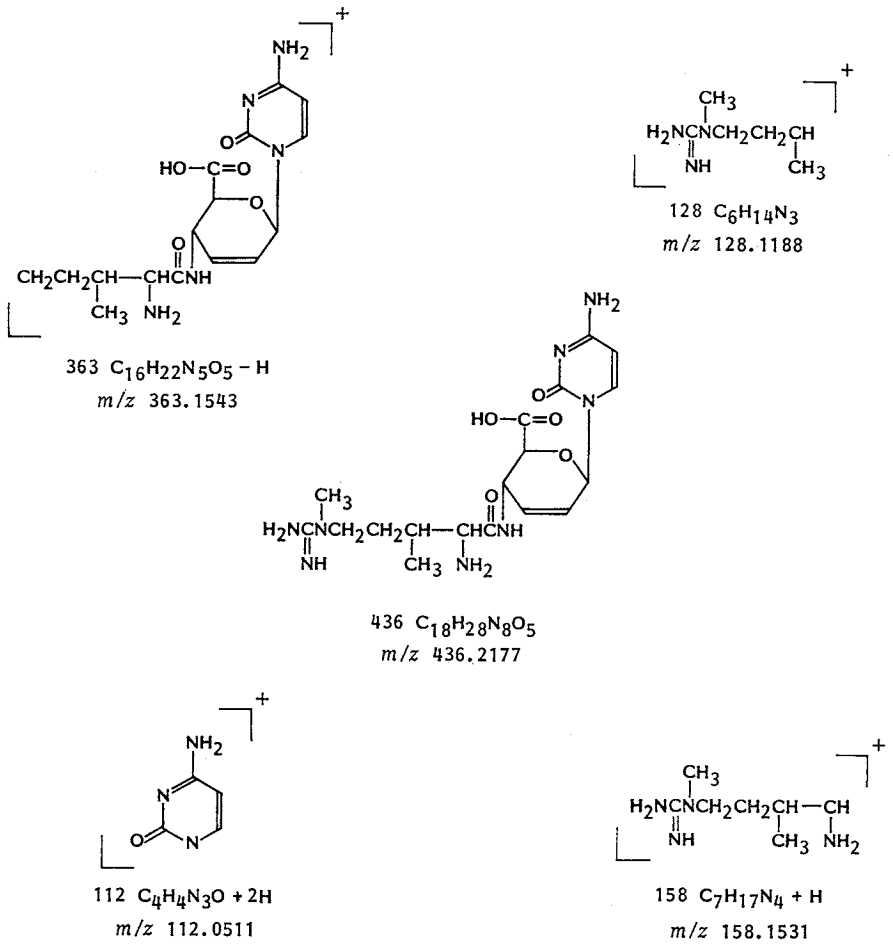
The electrophoretic mobility of arginomycin and its adsorption on both cationic and anionic exchange resins indicated the amphoteric nature of the antibiotic. This conclusion is supported by the IR spectra of arginomycin in its "zwitter" ionic form and its hydrochloride. Arginomycin shows IR absorptions at 3197, 1649 (assigned to a carboxylate ion), 1605, 1522, 1490, 1411, 1391, 1354, 1296, 1280, 1229, 1203, 1184, 1117, 1070, 826, 788, 776 and 721 cm^{-1}. Arginomycin hydrochloride, ($[\alpha]_D^{25}$ +40° (*c* 0.9, water); mp *ca.* 120°C (dec); UV λ_{max} nm (ε) 271 (7,950)), shows in addition to the above IR absorptions a carbonyl band at 1727 cm^{-1} assigned to a non-ionized carboxyl group. Furthermore treatment of arginomycin with methanolic hydrogen chloride yielded its methyl ester: $[\alpha]_D^{25}$ +15° (*c* 1.0, water); mp *ca.* 110°C (dec); UV λ_{max} nm (ε) 268 (5,950). HRFAB-MS spectrum (in the positive ion mode) of arginomycin methyl ester (Table 1) gave a $(M+H)^+$ peak at *m/z* 451.2412; calcd for $C_{19}H_{31}N_8O_5$, 451.2417, suggesting a molecular formula of $C_{19}H_{30}N_8O_5$ for arginomycin methyl ester. The IR spectrum of the methyl ester shows a strong ester carbonyl absorption band at 1736 cm^{-1} confirming the presence of a carboxyl group in the arginomycin molecule. Information on selected fragments observed in the FAB-MS of arginomycin is shown in Fig. 2. The significance of these fragments in relation to the structure of arginomycin is discussed later in this paper.

Table 1. Ions observed in the FAB-MS of arginomycin and its methyl ester.

Arginomycin		Arginomycin methyl ester
Positive ion mode	Negative ion mode	Positive ion mode
437 $(M+H)^+$	435 $(M-H)^-$	451 $(M+H)^+$
363	421	437
326	409	363
233	395	340
217	381	302
193	307	274
158	281	233
128	255	217
109	241	158
84	227	140
74		128
		112
		84
		70
		39

2-Hydroxyethyldisulfide (2-HED) was used as the matrix for both positive and negative ion mode FAB-MS.

Fig. 2. Fragments observed in the HRFAB-MS of arginomycin.



Arginomycin also contains strong basic group(s) as indicated by its electrophoretic behavior and strongly positive Sakaguchi and ninhydrin tests. The latter has been used for detection of the antibiotic and its degradation products on TLC plates. The positive Sakaguchi test combined with the presence of a singlet signal at δ 158.6 in the ^{13}C NMR spectrum of arginomycin support the presence of a guanidino group in the antibiotic.

The ^1H NMR spectra of arginomycin, arginomycin hydrochloride, and arginomycin methyl ester hydrochloride are presented in Table 2. A list of the carbon absorptions observed in the ^{13}C NMR spectrum of arginomycin and those reported for blasticidin S[2] is shown in Table 3. Both ^{13}C signals and the ^1H NMR absorptions observed are in agreement with the $C_{18}H_{28}N_8O_5$ molecular formula proposed for arginomycin on the basis of HRFAB-MS.

The Structure of Arginomycin

The structure of arginomycin is represented by **1a** (Fig. 1). The presence of a cytosine moiety in the antibiotic was suggested by UV spectra obtained in neutral, acidic, and basic aqueous solutions. This is supported by absorptions in the ^1H NMR spectrum at δ 6.09 (1H, d) and 7.63 (1H, d) assigned to H-5 and H-6 of the cytosine fragment and by ^{13}C NMR absorptions at δ 157.4 (s), 167.4 (s), 97.8

Table 2. Chemical shifts[a] observed in the ^1H NMR spectra of arginomycin, arginomycin hydrochloride and arginomycin methyl ester hydrochloride.

Assignment[b]	Chemical shift (δ), No. of protons, multiplicity[c]		
	Arginomycin	Arginomycin hydrochloride	Arginomycin methyl ester hydrochloride
H-5	6.09 (1H, d)	6.08 (1H, d)	6.08 (1H, d)
H-6	7.63 (1H, d)	7.68 (1H, d)	7.70 (1H, d)
H-1′	6.50 (1H, d)	6.41 (1H, d)	6.45 (1H, d)
H-2′	6.15 (1H, d)	6.10 (1H, d)	6.10 (1H, d)
H-3′	5.88 (1H, d)	5.75 (1H, d)	5.85 (1H, d)
H-4′	4.78 (1H, d)	4.60 (1H, d)	4.61 (1H, d)
H-5′	4.16 (1H, d)	4.09 (1H, d)	4.45 (1H, d)
H-2″	3.34 (1H, d)	3.81 (1H, d)	3.81 (1H, d)
H-3″	1.88 (1H, m)	1.98 (1H, m)	2.05 (1H, m)
H-4″	1.45 (1H) and	1.33 (1H) and	1.41 (1H) and
	1.71 (1H, m)	1.62 (1H, m)	1.70 (1H, m)
H-5″	3.3~3.5 (2H, m)	3.25 (2H, m)	3.28 (2H, m)
H-7″	3.03 (3H, s)	2.88 (3H, s)	2.90 (3H, s)
H-8″	1.02 (3H, d)	1.01 (3H, d)	0.98 (3H, d)
OCH_3	—	—	3.61 (3H, s)

[a] Relative to tetramethylsilane.
[b] For designation of protons, see Fig. 1.
[c] Multiplicity: s=singlet, d=doublet, m=multiplet.

Table 3. Chemical shifts[a] observed in the ^{13}C NMR spectra of arginomycin, arginomycoic acid and blasticidin S.

Carbon No.[b]	Chemical shift (δ), multiplicity[c]		
	Arginomycin	Arginomycoic acid	Blasticidin S
C-2	157.4 (s)	—	157.9 (s)
C-4	167.4 (s)	—	165.9 (s)
C-5	97.8 (d)	—	97.9 (d)
C-6	144.0 (d)	—	142.2 (d)
C-1′	80.9 (d)	—	80.6 (d)
C-2′	127.0 (d)	—	126.9 (d)
C-3′	134.6 (d)	—	133.7 (d)
C-4′	46.76 (d)	—	47.5 (d)
C-5′	79.10 (d)	—	78.5 (d)
C-6′	176.10 (s)	—	175.6 (s)
C-1″	175.8 (s)	179.86 (s)	171.7 (s)
C-2″	60.5 (d)	59.86 (d)	37.7 (t)
C-3″	35.25 (d)	33.49 (d)	47.5 (d)
C-4″	29.12 (t)	28.71 (t)	30.1 (t)
C-5″	49.81 (t)	48.63 (t)	47.4 (t)
C-6″	158.6 (s)	156.27 (s)	157.3 (s)
C-7″	36.9 (q)	35.56 (q)	36.7 (q)
C-8″	16.84 (q)	15.52 (q)	—

[a] Relative to tetramethylsilane.
[b] For numbering of carbons, see Fig. 1.
[c] Multiplicity in off-resonance decoupled spectra: s=singlet, d=doublet, t=triplet, q=quartet.

(d) and 144.0 (d) assigned to C-2, C-4, C-5 and C-6 of the cytosine moiety respectively.† The HRFAB-MS, (positive ion mode) of arginomycin contains a peak at *m/z* 326.1830 (calcd for $C_{14}H_{24}N_5O_4$, *m/z* 326.1828) resulting from the loss of a cytosine fragment, $C_4H_4N_3O$, from the molecular ion. Furthermore, the FAB-MS of arginomycin methyl ester hydrochloride contains a peak at *m/z* 112 $(C_4H_4N_3O+2H)^+$ as expected for cytosine resulting from fragmentation of a nucleoside.[3] Final confirmation for the presence of cytosine was obtained by isolation of this compound from acid hydrolysis of arginomycin. The obtained cytosine was identical in all respects to an authentic sample.

The presence of a pyran-3-ene moiety and its glycosidic linkage to cytosine in arginomycin are suggested by ^1H and ^{13}C NMR spectra. In the ^1H NMR spectrum of arginomycin (Table 2) an anomeric and two olefinic proton signals were observed at δ 6.50 (s-like, H-1′), 6.15 (d-like, J=10 Hz, *cis* coupling, H-2′) and 5.88 (d-like, J=10 Hz, H-3′). The presence of the "anomeric" (C-1′) carbon and the two olefinic carbons at C-2′ and C-3′ of arginomycin is supported by absorption at δ 80.9 (d), 127.0 (d) and 134.6 (d) in the ^{13}C NMR spectrum. Two dimensional homonuclear correlation (COSY) (Fig. 3) and specific proton decoupling experiments confirm the H-1′, H-2′, H-3′ relationships. In addition, results from COSY indicate the coupling of H-3′ to H-4′ (δ 4.78, 1H, d-like) which in turn is coupled to H-5′ (δ 4.16, 1H, d). The ^{13}C NMR chemical shifts for C-4′ (δ 46.76, d) and C-5′ (δ 79.10, d) are consistent with the pyran-3-ene moiety as indicated in **1a**. Furthermore, H-5′ which appears at δ 4.16 in the ^1H NMR spectrum of arginomycin and at δ 4.09 in that of arginomycin hydrochloride, shifts to δ 4.45 in the ^1H NMR of arginomycin methyl ester hydrochloride, indicating the attachment of the carboxyl group of arginomycin at C-5′ as shown in **1a**.

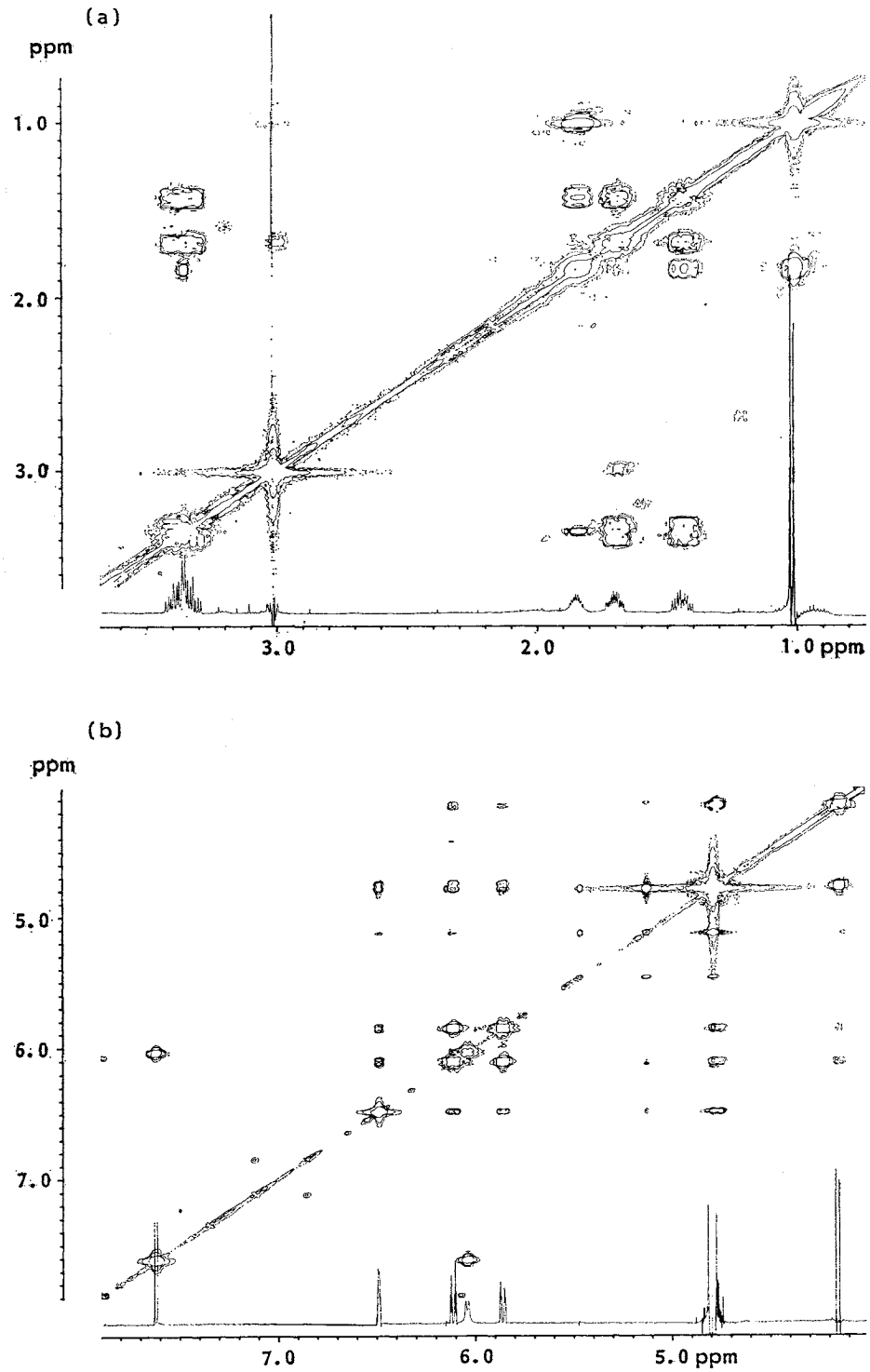
The data discussed thus far support a partial structure for arginomycin in which cytosine is linked to the pyran-3-ene carboxylic acid fragment as shown in Fig. 1. This nucleoside moiety is also present in blasticidin S, $C_{17}H_{26}N_8O_5$, an antifungal compound also active against plant pathogens[4,5] and has been designated as cytosinine.[6] Comparison of the ^{13}C NMR (Table 3) and ^1H NMR absorptions reported for blasticidin S or cytosinine†† to those observed for arginomycin establishes the identity of the nucleoside fragments present in both antibiotics.

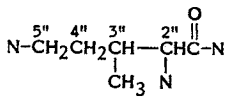
The remainder of the arginomycin molecule, $C_8H_{17}N_4O$, which can only be linked to the amino group at C-4′ of cytosinine, contains a guanidino group (positive Sakaguchi; ^{13}C NMR δ 158.6 (s)), an NCH_3 (^{13}C NMR δ 36.9 (q); ^1H NMR δ 3.03 (3H, s)), an $CHCH_3$ (^{13}C NMR δ 35.25 (d), 16.84 (q); ^1H NMR δ 1.88 (1H, m), 1.02 (3H, d), two methylene groups one of which must be attached to nitrogen (^{13}C NMR δ 29.12 (t), 49.81 (t)), one methine group also attached to nitrogen, possibly to a primary amino group (^{13}C NMR δ 60.25 (d, positive ninhydrin)) and most probably a peptide carbonyl carbon (^{13}C NMR δ 175.8 (s)). Two dimensional homonuclear correlation (Fig. 3a) indicated the presence of the carbon chain **5** in arginomycin. Consideration of the chemical shifts in both ^1H and ^{13}C NMR spectra of the groups present in **5** (Tables 2 and 3, Fig. 3) led to the postulation of structure **6** for the $C_8H_{17}N_4O$ fragment linked to cytosinine in arginomycin. This conclusion is supported by the HRFAB-MS data on arginomycin (**1a**) shown in Fig. 2. Ion peaks at *m/z* 128,

† Reported for cytosine: ^1H NMR δ 7.69 (H-6), 5.88 (H-5) in basic D_2O (Varian NMR Spectra Catalog, Varian Associates, 1962, Spectrum No. 402); δ 7.93 (H-6), 6.18 (H-5) in D_2O at pH 5.5.[10] ^{13}C NMR δ 157.0 (s, C-2), 166.7 (s, C-4), 95.7 (d, C-5), 142.8 (d, C-6).[11]

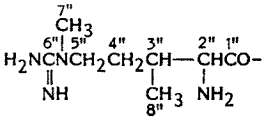
†† Reported for ^1H NMR of cytosinine, δ H-5, 6.16; H-6, 7.64; H-1′, 6.62; H-2′, 6.40; H-3′, 6.17; H-4′, 4.90; H-5′, 4.36.[2]

Fig. 3. Contour plot of a 2D correlated ^1H NMR of arginomycin.
(a) δ 1.0~4.0, (b) δ 4.0~8.0 (500 MHz, D_2O).





5



6

158 and 363 mass units with exact compositions of $(C_6H_{14}N_3)^+$, $(C_7H_{17}N_4+H)^+$ and $(C_{16}H_{22}N_5O_5-H)^+$, respectively, can only be explained by the structures shown in Fig. 2 confirming the relative positions of the guanidino, NH_2 and amide carbonyl groups as shown in **6**.

The placement of the NCH_3 at the guanidino nitrogen attached to C-5″ of **6** is based on the observed chemical shifts of the C-5″ carbons in the ^{13}C NMR spectra of arginomycin and blasticidin S (Table 3). Furthermore, **6** is a dimethyl-arginine. The ^{13}C NMR chemical shift of C-5 of arginine is reported to be δ 41.2.[7] Methylation of the guanidino group, as in **6**, is expected to result in a downfield shift of *ca.* 10 ppm for C-5″ while the presence of the methyl group at C-3″ of **6** is expected to result in an upfield shift of *ca.* 2 ppm with the net result of a downfield shift of *ca.* 8 ppm or a chemical shift of *ca.* δ 49.20 for C-5″ of **6**.[8] The observed chemical shift of δ 49.8 is in excellent agreement with the expected value. In addition reaction of arginomycin with 2,4-pentandione yielded a *N*-(4,6-dimethylpyrimidin-2-yl) derivative of arginomycin, **7**. The ^1H and ^{13}C NMR spectra of **7** show that the two methyl groups of the dimethylpyridinyl moiety appear as a singlet (^1H NMR δ 2.22 (6H, s); ^{13}C NMR δ 21.42 (C-2)) indicating that these groups have identical environments and, therefore, the NCH_3 is linked as shown in **6** or **7**.

It should be noted that the methylene proton absorptions due to 4″-CH_2 of **6** appear at δ 1.45 (1H) and 1.71 (1H) indicating non-equivalence of the methylene protons due to the asymmetry caused by the presence of a methyl group at C-3″ of **6**. The methylene protons at C-5″ (δ 3.3~3.5) are also non-equivalent; additional splitting by the adjacent methylene protons results in an unresolved multiplet.

Acid hydrolysis of arginomycin, as mentioned earlier, yielded cytosine and a $C_8H_{18}N_4O_2$ amino acid, designated arginomycoic acid, **2** (Fig. 1). The structure of arginomycoic acid was established by HRFAB-MS and ^1H and ^{13}C NMR spectra as described in the experimental and discussed in detail earlier in this section. Arginomycoic acid is a new amino acid with two asymmetric centers at C-2 and C-3, the stereochemistry of which is not known at present. An *N*-demethylarginomycoic acid-like amino acid has been reported to be present in the antibiotic lavendomycin reported recently.[9]

Work on the stereochemistry and biosynthesis of arginomycoic acid is under way. Preliminary results indicate that both the NCH_3 and CCH_3 groups present in arginomycoic acid are derived from C-1 donors and specifically the methyl group of methionine.

Biological Properties of Arginomycin

Arginomycin is characterized by low toxicity when administered intraperitoneally in mice, LD_{50}, 1,300 mg/kg. The antibiotic, which is weakly active vs. Gram-positive organisms and fungi, is under evalution in several disease areas.

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