ALTROMYCINS, NOVEL PLURAMYCIN-LIKE ANTIBIOTICS

II. ISOLATION AND ELUCIDATION OF STRUCTURE

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(Received for publication September 19, 1989)

A novel complex of Gram-positive antibiotics was produced from the fermentation of an actinomycete culture AB 1246E-26. The antibiotics were recovered from the whole fermentation broth by extraction with organic solvent and isolated using counter-current chromatography. UV and IR data place these compounds in the anthraquinone-derived class of antibiotics. Mass spectral and NMR data indicate a new complex of compounds related to, but distinctly different from the pluramycin type antibiotics.

In the course of screening soil microorganisms for activity against *Pseudomonas aeruginosa*, an actinomycete was discovered which produced four new related antibiotics. UV and IR data place these compounds in the anthraquinone-derived class of antibiotics, but their formulae have not been reported among members of this class. NMR studies indicate that these compounds are related to, but distinctly different from pluramycin type compounds. Previously reported members of the pluramycin class typically have amino sugars connected to carbons 8 and 10 of the D ring on the anthraquinone-γ-pyrone nucleus. The altromycins are novel in the occurrence of an unusual disaccharide unit on carbon 10 of the D ring while carbon 8 of the D ring is unsubstituted. The disaccharide unit consists of an amino sugar common to the pluramycins and a neutral *O*-glycosidic sugar not found in the pluramycins. An additional novel feature seen in these compounds is the substitution of carbon 13 on the B ring with a neutral *C*-glycoside. The altromycins differ from one another in the sugar regions of the molecule. The structural characterization of these compounds is outlined in this paper. Antibiotic production and microbiological data are presented in a companion paper[1].

Isolation

The whole fermentation broth was adjusted to pH 10.0 at harvest and extracted with methylene chloride. The organic extract was concentrated under reduced pressure and partitioned using a stationary droplet counter-current device. Further purification of the antibiotic complex was achieved with an Ito Coil Planet Centrifuge (CPC)[2]. Two standard combinations of solvents were used: 1) Methanol - water - carbon tetrachloride, 2) hexane - ethyl acetate - methanol - water. Activity was monitored by disc diffusion bioassay on agar plates seeded with *P. aeruginosa*.

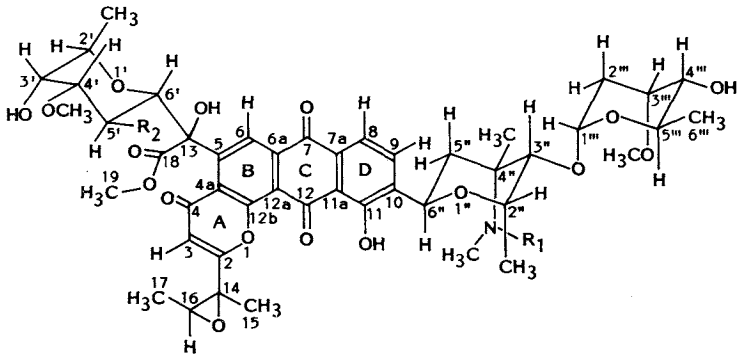
Characterization

The altromycins are orange-red compounds which are readily soluble in methanol, chloroform, carbon tetrachloride, dimethyl sulfoxide, ethyl acetate, benzene and dilute acid. The compounds are moderately soluble in 0.01 M phosphate buffer and only slightly soluble in *n*-hexane or water.

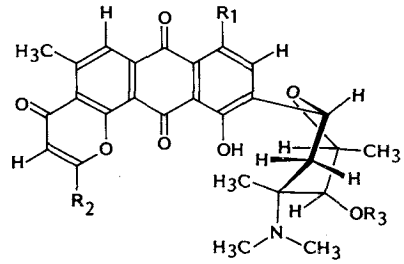
The altromycins (**1**~**4**) exhibit a complex, pH dependent, UV spectrum (Fig. 1). The definitive nature

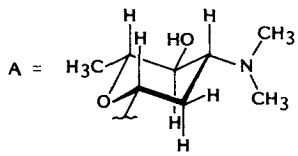
of these spectra, which were typical of that exhibited by 1-hydroxyanthraquinone-γ-pyrone (indomycinone) systems, was useful in defining the basic structural unit. The spectra are similar to those reported for pluramycin (**5**), neopluramycin (**6**), hedamycin (**7**) and kidamycin (**8**)[3~6]. The UV chromophores for altromycins A through D are identical.

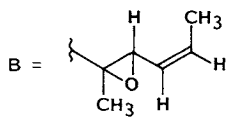
The IR spectra for altromycins A through D are essentially identical and are represented by that of altromycin B in Fig. 2. Diagnostic bands can be seen at 1748, 1674, 1649 and 1589 cm^{-1} ($CDCl_3$ thin film). The band at 1748 cm^{-1}, typical an aliphatic ester, is higher than that reported for the single acetate

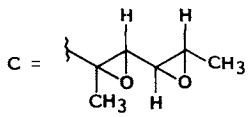


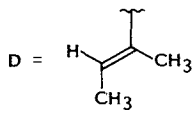
Altromycin A (**1**) $R_1=H$ $R_2=OH$
Altromycin B (**2**) $R_1=CH_3$ $R_2=OH$
Altromycin C (**3**) $R_1=H$ $R_2=H$
Altromycin D (**4**) $R_1=CH_3$ $R_2=H$

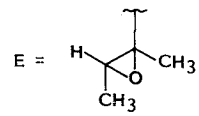












Pluramycin (**5**) $R_1=A$ $R_2=B$ $R_3=COCH_3$
Neopluramycin (**6**) $R_1=A$ $R_2=D$ $R_3=COCH_3$
Hedamycin (**7**) $R_1=A$ $R_2=C$ $R_3=H$
Kidamycin (**8**) $R_1=A$ $R_2=D$ $R_3=H$
14,16-Epoxykidamycin (**9**) $R_1=A$ $R_2=E$ $R_3=H$
Ankinomycin (**10**) $R_1=H$ $R_2=C$ $R_3=H$

ester of pluramycin (**5**) or neopluramycin (**6**) and suggests some other type of ester functionality.

HRFAB-MS in the positive ion mode gave an $(M+H)^+$ (peak matched) parent ion at *m/z* 912.3645 (calcd 912.3654) for altromycin A (**1**), indicating a molecular formula of $C_{46}H_{57}NO_{18}$. DCl-D_2O exchange experiments in a deuteroglycerol matrix gave a deuterated $(M+D)^+$ parent peak of 919, indicating six exchangeable protons in altromycin A. Altromycin B (**2**) gave an $(M+H)^+$ parent mass of 926.3828 (calcd 926.3810) indicating a molecular formula of $C_{47}H_{59}NO_{18}$ (five exchangeables). The $(M+H)^+$ parent mass of altromycin C (**3**) was 896.3686 (calcd 896.3705) indicating $C_{46}H_{57}NO_{17}$ (five exchangeables) and the $(M+H)^+$ for altromycin D (**4**) was 910.3868 (calcd 910.3861) indicating $C_{47}H_{59}NO_{17}$ (four exchangeables). These formulae set the altromycins apart from any of the known antibiotics of the pluramycin type.

Structure Determination

The UV/visible absorption spectra characterized the chromophore of the altromycins as an anthraquinone-γ-pyrone, similar to that of hedamycin (**7**) and kidamycin (**8**). This was confirmed by ^1H and ^{13}C NMR studies, which included HETCOR[7] and comparison with published data for hedamycin, kidamycin[8] and ankinomycin (**10**)[9]. In the ^1H NMR, characteristic singlets are seen at δ 6.5 (3-H) for the single proton on the pyrone (A) ring and δ 8.7 (6-H) for the single proton on the B ring. C-8 of the anthraquinone nucleus in the altromycins is not substituted as evidenced by the attached proton (in HETCOR data) which couples in an AB spin pattern to 9-H. This lack of substitution was without precedent until the recent

Fig. 1. UV spectra of altromycin B.

——MeOH, – – –1.0 N NaOH-95% MeOH, —·—1.0 N HCl-95% MeOH.

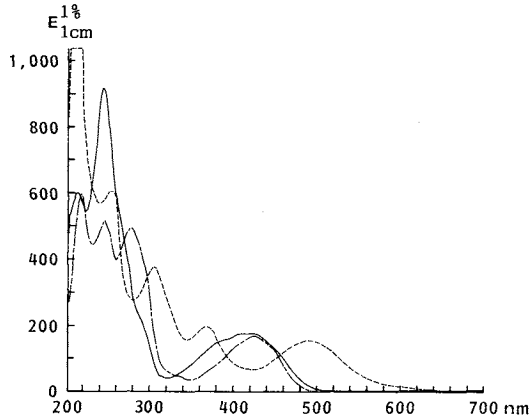


Fig. 2. IR spectrum of altromycin B.

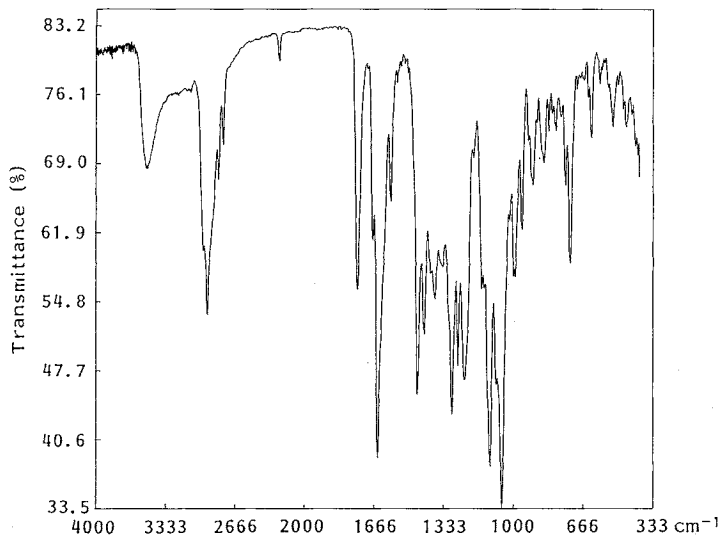
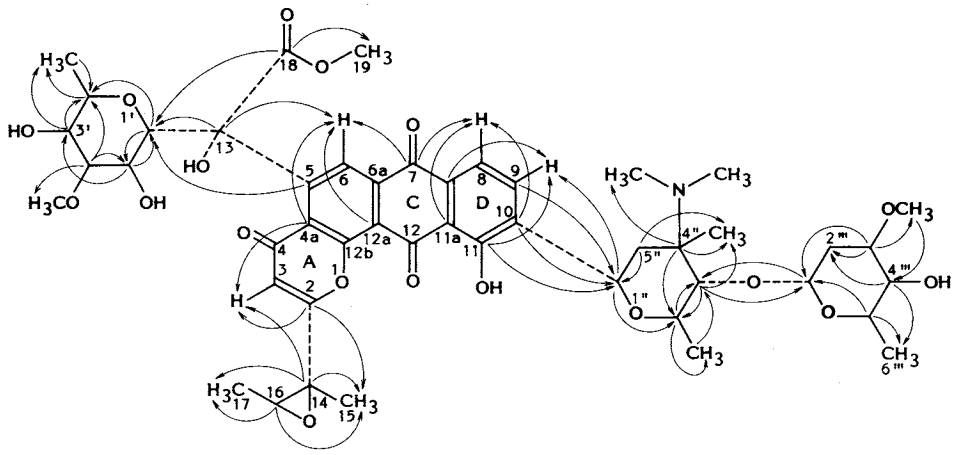


Table 1. ^{13}C NMR chemical shift assignments for altromycins A~D[a] and ankinomycin[b] in $CDCl_3$.

Carbon No.	Altromycin A (1)	Altromycin B (2)	Altromycin C (3)	Altromycin D (4)	DEPT[c]	Ankinomycin (8)
2	167.5	167.4	167.0	167.0	Q	166.3
3	111.1	110.9	110.9	110.8	CH	110.0
4	180.2	180.0	179.4	179.4	Q	178.8
4a	126.6	126.4	126.5	126.4	Q	126.5
5	149.2	149.1	149.4	149.2	Q	149.9
6	122.5	122.4	122.9	122.8	CH	126.0
6a	137.2	137.1	137.0	136.9	Q	136.3
7	181.2	181.0	181.2	181.2	Q	181.5
7a	130.3	130.2	130.4	130.3	Q	130.7
8	119.8	119.7	119.7	119.7	CH	119.3
9	133.6	133.7	133.4	133.6	CH	133.2
10	141.3	141.1	141.0	140.9	Q	140.7
11	159.1	159.2	159.3	158.9	Q	159.5
11a	115.8	115.6	115.9	115.7	Q	116.1
12	186.9	186.9	187.1	186.9	Q	187.7
12a	121.8	121.6	121.6	121.6	Q	119.8
12b	156.8	156.7	156.6	156.5	Q	156.2
13	80.9	80.8	79.0	78.9	Q	(24.1)[d]
14	59.8	59.7	59.8	59.7	Q	57.7
15	19.7	19.6	19.8	19.7	CH_3	14.4
16	62.7	62.5	62.5	62.5	CH	63.9
17	13.4	13.3	13.4	13.3	CH_3	(55.4)
18	170.5	170.4	170.9	170.9	Q	(51.8)
19	52.6	52.5	52.4	52.3	CH_3	(17.2)
2′	73.8	73.8	74.9	74.9	CH	—
3′	68.9	68.9	68.5	68.2	CH	—
4′	80.2	80.1	74.8	74.9	CH	—
5′ (**1** & **2**)	67.9	67.9	—	—	CH	—
5′ (**3** & **4**)	—	—	26.1	26.0	CH_2	—
6′	73.7	73.6	70.3	70.2	CH	—
2′-CH_3	14.1	14.0	14.7	14.7	CH_3	—
4′-OCH_3	57.9	57.8	55.6	55.5	CH_3	—
2″	70.3	70.7	70.2	70.8	CH	67.9
3″	77.7	82.6	77.8	82.7	CH	71.0
4″	54.9	58.1	55.1	58.1	Q	57.7
5″	40.3	44.7	40.4	44.7	CH_2	35.0
6″	62.1	62.2	62.3	62.2	CH	68.2
2″-CH_3	14.7	13.5	14.8	13.5	CH_3	17.5
4″-CH_3	24.1	14.0	23.8	13.9	CH_3	13.2
4″-$N(CH_3)_n$	27.9	40.3	27.8	40.3	CH_3	37.1
1‴	93.4	94.4	93.7	94.5	CH	—
2‴	30.8	31.1	30.9	31.1	CH_2	—
3‴	74.8	74.9	74.8	74.9	CH	—
4‴	72.1	72.1	72.1	72.0	CH	—
5‴	65.4	65.0	65.7	65.0	CH	—
6‴	17.7	17.6	17.7	17.6	CH_3	—
3‴-OCH_3	55.9	56.1	56.1	56.1	CH_3	—

[a] Measured at 75 or 125 MHz; chemical shifts in ppm from TMS.
[b] Measured at 100 MHz[9].
[c] ^{13}C NMR DEPT data for altromycins.
[d] Non-comparable carbons.
n: (**1**), 1; (**2**), 2; (**3**), 1; (**4**), 2; (**8**), 2.

Fig. 3. Long range HETCOR data summary for altromycins A and B.



description of ankinomycin; all previously described pluramycins carry a *C*-glycosidically bound angolosamine moiety at C-8. Direct comparison of carbon resonances with published data, with appropriate allowances for the lack of substitution at C-8 and the structural elaboration at C-13 enabled assignment of the carbons of the chromophore (Table 1). These were supported by multiplicity analysis using the DEPT technique and by one bond and long range HETCOR data (Fig. 3). A second major structural variation from known pluramycins is evident by the lack of a methyl carbon corresponding to C-13 which shows resonance at *ca.* 24 ppm. In contrast, this carbon in the altromycins is assigned as a quaternary carbon at approximately 80 ppm. This carbon is presumably derived from the C-2 carbon of the ultimate acetate unit in the polyketide precursor. This is supported by a single precedent for the altromycin type structure in which the carboxyl carbon of that unit has been retained in the final product (antibiotic SS43405D)[10]. The nature of substituents at C-13 in the altromycins will be discussed after consideration of the sugar moieties. Despite the absence of angolosamine on C-8 in the altromycins, the molecular formula indicates a larger molecule than the common pluramycins. The altromycins show a common loss of 144 amu in FAB-MS (positive ion mode) with resultant fragment ions indicating the loss of a neutral sugar. These fragment ions are in the molecular weight range of previously reported pluramycin compounds (~688~770 amu)[11], suggesting a more complex structure for the altromycins (895~925 amu).

The long range HETCOR experiments along with ^{13}C NMR DEPT data enabled the construction of three separate sugar moieties associated with the altromycin chromophore. The HETCOR data indicated two neutral sugars and *N,N*-dimethylvancosamine (*N*-methylvancosamine is found in altromycins A and C). The neutral sugars were shown to have anomeric and pseudoanomeric carbons at *ca.* 94 ppm (C-1‴) and 73 ppm (C-6′), indicating an *O*-glycosyl and *C*-glycosyl linkage, respectively. The sugar *N,N*-dimethylvancosamine, normally found in the pluramycins at C-10 on the chromophore, was also found to be at this location in the altromycins (evidenced by a HETCOR coupling: C-6″ to 9-H and C-9, C-10, C-11 to 6″-H). A strong HETCOR coupling between the anomeric C-1‴ of the neutral *O*-glycosidic sugar and 3″-H of the vancosamine moiety indicates that these two units are linked as a novel disaccharide at C-10. The quaternary C-13 near 80 ppm showed HETCOR couplings to both 6-H of the chromophore and 6′-H of the other *C*-glycosidic sugar moiety. In addition, a 2D *J* correlated map experiment (COSY) demonstrated a long range coupling between 6-H and 6′-H. HETCOR couplings to 6′-H were also seen

Table 2. ^{1}H NMR chemical shift assignments and coupling data for altromycins A~D in $CDCl_3$.

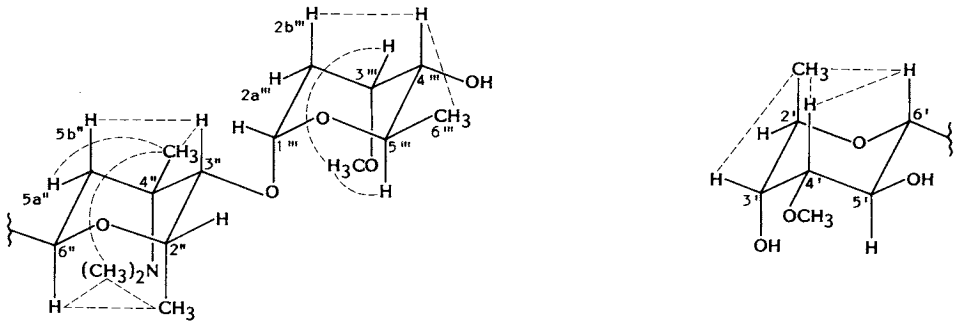
Position	Altromycin A (1)			Altromycin B (2)			Altromycin C (3)			Altromycin D (4)		
	δ	m[a]	J, Hz	δ	m	J, Hz	δ	m	J, Hz	δ	m	J, Hz
3	6.55	s	—	6.52	s	—	6.53	s	—	6.51	s	—
6	8.72	s	—	8.69	s	—	8.85	s	—	8.85	s	—
8	7.87	d	7.8	7.85	d	8.0	7.87	d	7.7	7.86	d	7.9
9	7.98	d	7.8	7.98	d	8.0	7.97	d	7.7	7.99	d	7.9
11-OH	13.15	s	—	13.17	s	—	13.17	s	—	13.20	s	—
15	1.94	s	—	1.93	s	—	1.93	s	—	1.93	s	—
16	3.37	q	5.4	3.35	q	5.4	3.36	q	5.4	3.36	q	5.4
17	1.28	d	5.4	1.25	d	5.4	1.29	d	5.4	1.28	d	5.4
19	3.72	s	—	3.69	s	—	3.69	s	—	3.69	s	—
2′	4.33	dq	7.3, 1.3	4.31	dq	7.3, 1.7	4.40	dq	7.3, 1.6	4.40	dq	7.1, 1.6
3′	3.97	dd	3.3, 1.3	3.96	dd	3.2, 1.7	3.83	dd	2.9, 1.6	3.83	dd	3.0, 1.6
4′	3.61	dd	8.7, 3.3	3.59	dd	8.7, 3.2	3.68	dd	9.9, 2.9	3.68	dd	9.9, 3.0
5a′	—	—	—	—	—	—	2.06	br		2.06	br	
5b′	4.69	dd	9.0, 8.7	4.66	dd	9.0, 8.7	2.04	m		2.04	m	
6′	4.59	d	9.0	4.56	d	9.0	4.80	d	10.0	4.79	d	10.0
2′-CH_3	1.75	d	7.3	1.73	d	7.3	1.66	d	7.3	1.65	d	7.1
4′-OCH_3	3.62	s	—	3.60	s	—	3.42	s	—	3.43	s	—
2″	4.44	dq	6.9, 6.0	4.32	dq	6.9, 5.7	4.42	dq	6.9, 6.0	4.34	dq	6.8, 5.7
3″	3.81	d	6.0	3.77	d	5.7	3.80	d	6.0	3.77	d	5.7
5a″	2.40	d	14.1	2.48	d	13.5	2.40	d	13.9	2.49	d	14.7
5b″	1.28	dd	14.1, 10.7	1.25	dd	13.5, 10.3	1.23	dd	13.9, 10.5	1.24	dd	14.7, 11.1
6″	5.50	d	10.7	5.53	d	10.3	5.50	d	10.5	5.55	d	11.1
2″-CH_3	1.58	d	6.9	1.65	d	6.9	1.56	d	6.9	1.68	d	6.8
4″-CH_3	1.26	s	—	1.04	s	—	1.22	s	—	1.05	s	—
4″-$N(CH_3)_n$	2.47	s	—	2.47	s	—	2.47	s	—	2.47	s	—
1‴	4.89	d	3.7	4.79	d	4.4	4.89	d	3.9	4.80	d	4.4
2a‴	2.31	dd	14.9, 3.5	2.31	dd	14.9, 3.1	2.31	dd	14.9, 3.2	2.32	dd	14.9, 3.3
2b‴	1.78	ddd	14.9, 3.7, 3.6	1.78	ddd	14.9, 4.4, 3.2	1.79	ddd	14.9, 3.9, 3.0	1.78	ddd	14.9, 4.4, 3.0
3‴	3.67	ddd	3.6, 3.5, 3.2	3.62	ddd	3.5, 3.2, 3.1	3.68	ddd	3.5, 3.2, 3.0	3.63	ddd	3.5, 3.3, 3.0
4‴	3.30	dd	9.3, 3.2	3.27	dd	9.6, 3.5	3.26	dd	9.5, 3.5	3.27	dd	9.6, 3.5
5‴	4.05	dq	9.3, 6.4	4.09	dq	9.6, 6.3	4.05	dq	9.5, 6.4	4.09	dq	9.6, 6.2
6‴	1.29	d	6.4	1.26	d	6.3	1.26	d	6.4	1.26	d	6.2
3‴-OCH_3	3.47	s	—	3.40	s	—	3.48	s	—	3.42	s	—

[a] m: Multiplicity.
n: (1), 1; (2), 2; (3) 1; (4), 2.

from the carbonyl of a methyl ester and a quaternary carbon near 149 ppm, C-5 of the chromophore. These data assigned C-13 to the role of a bridging carbon between the chromophore and the neutral *C*-glycosidic sugar which is substituted with a methyl ester group. Chemical shift along with molecular formula considerations lead to a hydroxyl group also being placed on this carbon.

The quaternary carbon (C-14) of a simple dimethyl epoxide side chain exhibited a HETCOR coupling to 3-H of the pyrone (A) ring. In addition, couplings were seen between 16-H/15-CH_3 of the epoxide and 3-H of the pyrone ring in the COSY experiment. These would indicate the point of attachment of the side chain to be the same as that found in pluramycin type compounds. The side chain is structurally similar to that found in 14,16-epoxykidamycin (**9**), the largomycin FII chromophore[12]. However, a 2D ^{1}H NOE in a rotating frame (NOE-CAMEL)[13] experiment, which shows an NOE cross peak between 16-H and 15-CH_3 and the absence of a cross peak between the protons of the two methyl groups 15-CH_3 and 17-CH_3, strongly suggests that these methyl groups of the epoxide side chain may be in the *trans*-orientation,

Fig. 4. Internal NOE-CAMEL data summary for sugar units on altromycins A and B.



opposite that implied in reference 12 for the structure of 14,16-epoxykidamycin. This possible difference in stereochemisty is supported by differences in the ^{13}C chemical shifts for carbons C-14 to C-17 ($\Delta\delta$ 2.2, 5.9, 0.7, 0.7, respectively) of 14,16-epoxykidamycin (**9**) and altromycin A (**1**); differences greater than might be expected due to the distal structural variances in these two molecules.

In addition to single frequency decoupling and total ^1H-correlation spectroscopy (TOCSY) experiments, a combination of COSY and 2D-TOCSY[14] data layed out in different colors on the same correlation map were used in determining the sequence of coupling systems for each sugar. Coupling constant and NOE data were used to determine the relative configuration of each sugar (Table 2).

The neutral *O*-glycosyl moiety attached to the amino sugar is common to altromycins A through D. This sugar contains two geminally coupled protons attached to the carbon near δ 30 ($J_{2a'''\text{-}2b'''}=14$ Hz) and two axial-axial vicinally coupled protons, 4'''-H and 5'''-H ($J_{4'''\text{-}5'''}=9$ Hz). 4'''-H shows a cross peak to 2b'''-H in the NOE-CAMEL experiment, indicating that these protons are orientated 1-3 axially to each other (Fig. 4). 5'''-H and 3'''-OCH_3 show the same type of 1-3 diaxial NOE. Taking into account that $J_{2b'''\text{-}3'''}=3$ Hz and $J_{3'''\text{-}4'''}=3$ Hz, this neutral *O*-glycosidic sugar was defined as 2,6-dideoxy-3-*O*-methylaltrose.

The amino sugar in the altromycins was shown to be the same as that commonly reported for the pluramycins; *N*,*N*-dimethylvancosamine. This sugar, in the altromycin series, is found in a chair conformation based on coupling constants and NOE data. A vicinal axial-axial coupling of $J=10$ Hz is exhibited between the doublet at δ 5.5 (6''-H) and one of the two geminal protons 5b''-H ($J_{5a''\text{-}5b''}=14$ Hz). A 1-3 diaxial cross peak is seen in the NOE-CAMEL experiment between 5b''-H and 3''-H. Additionally there is a 1-3-5 triaxial series of cross peaks between 6''-H, 2''-CH_3 and the amino methyls of the vancosamine moiety. These data indicate that although the configuration of this sugar is the same as that reported for hedamycin (**7**) and kidamycin (**8**)[15], it is in the chair conformation rather than the boat. This is presumably due to the substitution of C-3'' with the glycosyloxy group. The degree of substitution of the amine on this sugar varies within the altromycins from monomethylated (altromycins A and C) to dimethylated (altromycins B and D).

The *C*-glycosyl moiety attached to C-13 exhibits NOE-CAMEL cross peaks from the pseudoanomeric 6'-H to both 4'-H and 2'-CH_3 indicating a 1-3-5 triaxial relationship. The coupling constants $J_{4'\text{-}5'}=9$ Hz and $J_{5'\text{-}6'}=9$ Hz lead to the assignment of proton positions 4-6 as axial-axial-axial. With this information and in consideration that $J_{2'\text{-}3'}=2$ Hz, $J_{3'\text{-}4'}=3$ Hz and an NOE is seen between 3'-H and 2'-CH_3, this sugar was shown to be the *C*-glycoside of 6-deoxy-3-*O*-methylaltrose in altromycins A and B. In altromycins

C and D, C-5′ is found as a CH_2 near 26 ppm in the ^{13}C NMR DEPT and HETCOR experiments (C-5′ is *ca.* 67 ppm CHOH in altromycins A and B). This accounts for the one less exchangeable proton found in altromycins C and D than in altromycins A and B. The neutral *C*-glycosyl moiety in altromycins C and D is therefore an analog of 2,6-dideoxy-3-*O*-methylaltrose. Although the structures depicted in **1** through **4** show the sugars in the absolute stereochemistry as would be expected from D-glucose, no data to support this is available; nor is any data available on the absolute stereochemistry of carbons 13, 14 and 16.

The structures of the novel compounds altromycins A through D were determined to be that of **1**～**4** based on analysis of the spectroscopic data presented in this paper. The altromycins are set apart from previously reported pluramycin-type compounds by virtue of a unique vancosamine-altrose analog disaccharide unit at the C-10 position of the chromophore, while C-8 of the chromophore is unsubstituted. An additional novel feature is seen in the presence of a *C*-glycoside analog of altrose attached to C-13 of the chromophore, found as both the 5′-hydroxy and 5′-deoxy version.

Experimental

General Procedures

UV spectra were recorded on a Perkin-Elmer Lambda 3B UV/VIS spectrophotometer, and IR spectra on a Perkin-Elmer 683 dual beam dispersive instrument. Optical rotations were measured in a 10-cm tube on a Perkin-Elmer model 241 polarimeter. Mass spectra were recorded on a Kratos MS-50 spectrometer in the FAB mode. Using TMS as an internal standard, NMR spectra were aquired employing a General Electric GN300 or GN500 spectrometer. ^1H NMR data were acquired at 300 or 500 MHz in $CDCl_3$. ^{13}C NMR data were taken at 75 or 125 MHz in $CDCl_3$.

Isolation of the Altromycins

Whole broth (18 liters), adjusted to pH 10.0 with NH_4OH, was extracted three times with 4 liter portions of methylene chloride. The methylene chloride extracts were combined and concentrated under reduced pressure on a vertical evaporator to approximately 4 g of oil. The concentrate was partitioned in the solvent system MeOH - H_2O - CCl_4 (5 : 2 : 5) using a Square Coil Droplet Counter-Current device (made in-house) with the lower phase as the stationary. This device consists of 30 m of continuous 3/16″ ID teflon tubing wrapped in a 2 × 14 cm repeating pattern around an oak board. The coil has 96 turns with a total internal volume of 450 ml. Approximately one half volume is retained as stationary phase (by Gravity). Material with substantial activity against *P. aeruginosa* was eluted with a flow rate of 5 ml/minute in fractions 26～80 (10～12 ml) which yielded approximately 255 mg and fractions 117～121 (10～12 ml, beginning of stationary phase blow-out) which contained 570 mg. Both activities were concentrated to orange-red oils.

The activity from fractions 26～80 was partitioned on a CPC counter-current device employing the following conditions: MeOH - H_2O - CCl_4 (lower phase stationary, 5 : 2 : 5), tail inlet, 4 ml/minute @800 rpm, 85～90% stationary retention, 10 ml fractions. Two areas of activity; fractions 35～41 (32.0 mg) and 71～105 (224.7 mg) were obtained. The activity from 71～105 was rechromatographed on the CPC under the following conditions: MeOH - 0.01 M NH_4OAc - CCl_4 (lower phase stationary, 5 : 2 : 5), tail inlet, 4 ml/minute @800 rpm, 85～90% stationary retention, 10 ml fractions. Two areas of activity were obtained: 16～21 (24.7 mg, same compound as in 35～41) and 48～80 (230.4 mg). Both were orange-red oils. Fractions 16～21 (35～41) contained altromycin A and fractions 48～80 altromycin B.

The activity from fractions 117～121 was partitioned on a CPC counter-current device employing the following conditions: Hexane - EtOAc - MeOH - H_2O (lower phase stationary, 3 : 7 : 6 : 4), tail inlet, 4 ml/minute @900 rpm 85～90% stationary retention, 8 ml fractions. One area of activity, fractions 37～45 was combined to yield 29.7 mg of orange-red oil. These fractions contained altromycins C and D. They were chromatographed using the CPC under the following conditions: Hexane - EtOAc - MeOH - 0.01 M NH_4OAc (lower phase stationary, 3 : 7 : 6 : 4), tail inlet, 4 ml/minute @900 rpm, 85～90% stationary retention, 10 ml fractions. Two areas of activity were obtained: 113～121 contained 6.2 mg of altromycin

D and 126~159 contained 2.5 mg of altromycin C.

Altromycin A: 912 $(M+H)^+$ FAB-MS, $C_{46}H_{57}NO_{18}$, $[\alpha]_D^{22}+80°$ (*c* 0.1, MeOH), altromycin B: 926 $(M+H)^+$ FAB-MS, $C_{47}H_{59}NO_{18}$, $[\alpha]_D^{22}+106°$ (*c* 0.08, MeOH), altromycin C: 896 $(M+H)^+$ FAB-MS, $C_{46}H_{57}NO_{17}$, altromycin D: 910 $(M+H)^+$ FAB-MS, $C_{47}H_{59}NO_{17}$, $[\alpha]_D^{22}+78°$ (*c* 0.1, MeOH).

UV absorption maxima in MeOH: λ_{max}^{MeOH} nm (ε) 212 (34,700), 242 (43,900), 290 (sh, 16,000), 385 (sh, 9,330), 408 (sh, 10,500), 424 (10,800); $\lambda_{max}^{1.0\,N\ NaOH\text{-}MeOH}$ nm (ε) 252 (34,800), 304 (21,400), 368 (13,200), 490 (12,300); $\lambda_{max}^{1.0\,N\ HCl\text{-}MeOH}$ nm (ε) 215 (34,200), 243 (29,400), 276 (28,200), 424 (10,500).

Acknowledgment

The authors express appreciation to RICHARD F. ANDERSON for detailed IR analysis.

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