Notes

ALTROMYCINS E, F, G, H AND I; ADDITIONAL NOVEL COMPONENTS OF THE ALTROMYCIN COMPLEX

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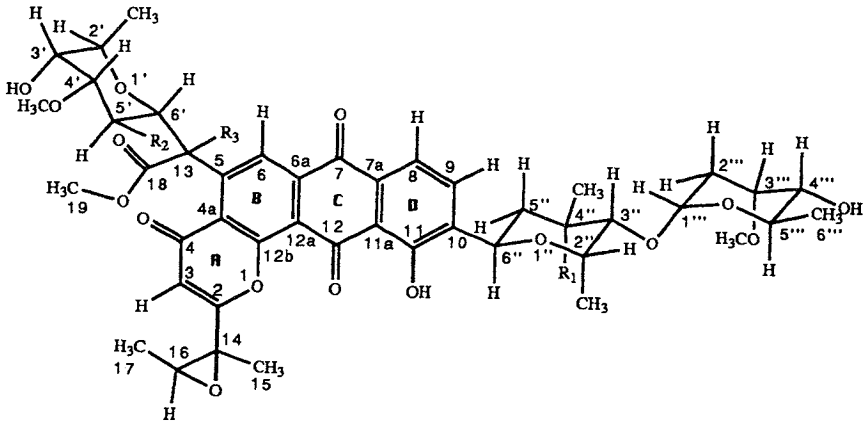
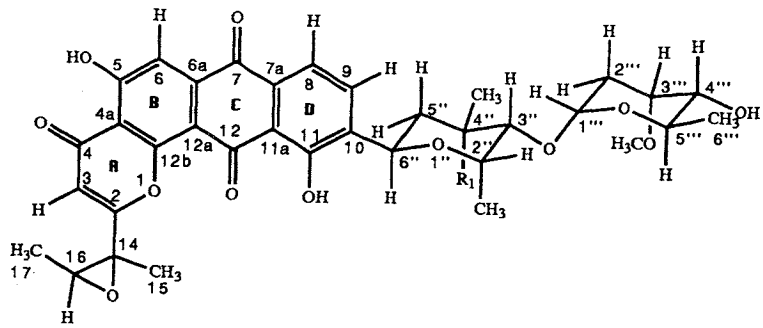
The altromycins are a novel complex of anthraquinone-derived Gram-positive antibiotics with potent antitumor activity. These compounds are produced by an actinomycete, AB 1246E-26 (NRRL 18371), obtained from a South African bushveld soil.[1] Altromycins A, B, C, and D, compounds structurally related to the pluramycin-group of antibiotics, have been previously reported as has some biological evaluations of the more abundant members.[2,3] SUN *et al.*, has demonstrated that altromycin B forms an intercalated threaded drug-DNA complex that is covalently bound through N7 of guanine in the major groove and has proposed a prototypic DNA adduct structure for the altromycin-pluramycin antitumor antibiotics.[4]



Altromycin A	$R_1=NHCH_3$	$R_2=OH$	$R_3=OH$
Altromycin B	$R_1=N(CH_3)_2$	$R_2=OH$	$R_3=OH$
Altromycin C	$R_1=NHCH_3$	$R_2=H$	$R_3=OH$
Altromycin D	$R_1=N(CH_3)_2$	$R_2=H$	$R_3=OH$
Altromycin E	$R_1=NHCH_3$	$R_2=OH$	$R_3=H$
Altromycin F	$R_1=N(CH_3)_2$	$R_2=OH$	$R_3=H$
Altromycin G	$R_1=NH_2$	$R_2=OH$	$R_3=OH$



Altromycin H	$R_1=NHCH_3$
Altromycin I	$R_1=N(CH_3)_2$

† Deceased.

After the discovery of altromycins A~D, subsequent fermentations of AB 1246E-26 produced five additional novel minor congeners, designated altromycins E~I. In this communication the isolation and structural characterization of altromycins E, F, G, H and I is presented. Altromycins E and F are deoxy analogs of A and B, respectively (Fig. 1); lacking the hydroxyl at the C-13 position. Altromycin G is the primary amine with otherwise the same structural features as altromycins A and B. Altromycins H and I contain more substantial structural changes, in that C-5 on the B ring of the anthraquinone-γ-pyrone nucleus carries a simple phenolic hydroxyl rather than the ester and C-glycoside substituents which are characteristic of the previously reported altromycins.

Several fermentations, ranging in size from 60 to 150 liters, were carried out as previously described.[1] Altromycins were isolated from each fermentation in the manner described here. Similar fractions were combined where it was appropriate. Whole fermentation broth was adjusted to pH 10.0 and a 25% volume of acetone was added with stirring. After standing for two hours the mixture was extracted with four 25% volumes of methylene chloride. The solvent extracts were concentrated and the sample was partitioned between equal volumes of methanol and *n*-heptane, and the methanol layer was partitioned twice more with *n*-heptane. The concentrated methanol layer was partitioned in a

Table 1. Data from high resolution fast atom bombardment mass spectrometry in the positive ion mode for altromycins E~I.

Altromycin	Molecular formula	$(M+H)^+$ Measured	$(M+H)^+$ Calculated
E	$C_{46}H_{57}NO_{17}$	896.3722	896.3705
F	$C_{47}H_{59}NO_{17}$	910.3832	910.3861
G	$C_{45}H_{55}NO_{18}$	898.3497	898.3497
H	$C_{36}H_{41}NO_{12}$	680.2712	680.2707
I	$C_{37}H_{43}NO_{12}$	694.2857	694.2863

Table 2. UV adsorption data for altromycins E~I.

Altromycin	Condition	Wavelength (Extinction coefficient)			
E	λ_{max}^{MeOH} nm	241	244	270 sh	421
	(ε)	(28,200)	(39,400)	(21,000)	(7,600)
	$\lambda_{max}^{1.0\,N\,HCl\text{-}MeOH}$ nm	216	244	270 sh	421
	(ε)	(24,200)	(41,700)	(21,500)	(8,100)
	$\lambda_{max}^{1.0\,N\,NaOH\text{-}MeOH}$ nm	251	340	556	
	(ε)	(34,900)	(8,100)	(3,600)	
F	λ_{max}^{MeOH} nm	215	245	270 sh	423
	(ε)	(28,200)	(40,000)	(21,800)	(7,300)
	$\lambda_{max}^{1.0\,N\,HCl\text{-}MeOH}$ nm	218	245	270 sh	423
	(ε)	(21,800)	(41,400)	(21,800)	(8,200)
	$\lambda_{max}^{1.0\,N\,NaOH\text{-}MeOH}$ nm	252	339	558	
	(ε)	(35,500)	(8,600)	(4,100)	
G	λ_{max}^{MeOH} nm	215	245	424	
	(ε)	(28,700)	(35,900)	(6,700)	
	$\lambda_{max}^{1.0\,N\,HCl\text{-}MeOH}$ nm	218	244	272	424
	(ε)	(29,200)	(29,200)	(22,000)	(8,100)
	$\lambda_{max}^{1.0\,N\,NaOH\text{-}MeOH}$ nm	252	302	368	497
	(ε)	(28,300)	(13,900)	(6,700)	(4,000)
H	λ_{max}^{MeOH} nm	217	247	278	423
	(ε)	(30,200)	(24,800)	(21,800)	(7,800)
	$\lambda_{max}^{1.0\,N\,HCl\text{-}MeOH}$ nm	217	245	278	423
	(ε)	(28,900)	(26,800)	(25,800)	(9,200)
	$\lambda_{max}^{1.0\,N\,NaOH\text{-}MeOH}$ nm	254	306	368	487
	(ε)	(27,200)	(20,400)	(11,200)	(8,200)
I	λ_{max}^{MeOH} nm	214	243	276	422
	(ε)	(36,100)	(28,800)	(25,000)	(9,400)
	$\lambda_{max}^{1.0\,N\,HCl\text{-}MeOH}$ nm	215	243	276	422
	(ε)	(34,700)	(29,100)	(28,800)	(10,400)
	$\lambda_{max}^{1.0\,N\,NaOH\text{-}MeOH}$ nm	252	305	368	485
	(ε)	(30,200)	(24,600)	(13,500)	(10,400)

countercurrent distribution using 450 ml aliquots of each phase of a biphasic mixture of MeOH-H_2O-CCl_4 (5:2:5). The combined lower phases from six passages through four stations were concentrated to yield a semisolid brown-red material. This material was chromatographed over Silica Gel 60 G (Merck)

Table 3. Tabulation of ^{13}C NMR data for altromycins E~I[a].

Carbon No.	E	F	G	H	I	Carbon type[b]
2	165.7	165.7	167.5	169.3	169.3	Q
3	111.3	111.3	111.1	109.1	109.1	CH
4	178.5	178.6	180.2	182.4	182.5	Q
4a	126.3	126.3	126.6	113.3	113.3	Q
5	148.2	148.2	149.3	166.7	166.7	Q
6	124.1	124.0	122.5	110.7	110.7	CH
6a	136.9	136.9	137.2	139.8	139.9	Q
7	181.5	181.4	181.2	180.8	180.8	Q
7a	130.5	130.5	130.5	130.6	130.4	Q
8	119.5	119.5	119.8	119.4	119.5	CH
9	133.3	133.5	133.7	132.4	132.8	CH
10	141.0	140.9	140.7	140.2	140.9	Q
11	159.3	159.3	159.4	159.1	159.1	Q
11a	115.9	115.9	115.9	115.6	115.4	Q
12	187.5	187.4	186.9	186.2	186.3	Q
12a	120.8	120.8	121.8	112.3	112.4	Q
12b	156.1	156.1	156.8	156.6	156.6	Q
13	—	—	80.9	—	—	Q
13	48.3	48.2	—	—	—	CH
14	59.8	59.7	59.8	60.0	60.0	Q
15	20.0	19.9	19.6	19.6	19.7	CH_3
16	62.5	62.5	62.7	62.7	62.7	CH
17	13.5	13.4	13.3	13.2	13.2	CH_3
18	170.4	170.4	170.5	—	—	Q
19	52.3	52.3	52.6	—	—	CH_3
2′	74.3	74.2	73.8	—	—	CH
3′	68.8	68.8	69.0	—	—	CH
4′	81.5	81.4	80.2	—	—	CH
5′	68.0	67.9	68.0	—	—	CH
5′	—	—	—	—	—	CH_2
6′	73.9	74.0	73.7	—	—	CH
2′-CH_3	14.7	14.6	14.0	—	—	CH_3
4′-OCH_3	57.1	57.1	58.0	—	—	CH_3
2″	70.3	70.8	70.0	70.0	70.7	CH
3″	77.8	82.8	76.0	77.3	82.8	CH
4″	54.9	58.1	51.7	56.1	58.1	Q
5″	40.4	44.8	44.9	38.5	44.8	CH_2
6″	62.2	62.3	62.3	63.6	62.3	CH
2″-CH_3	14.7	13.6	14.1	15.4	13.6	CH_3
4″-CH_3	24.2	14.1	32.6	22.6	14.0	CH_3
4″-$N(CH_3)_n$	28.1	40.4	—	27.2	40.3	CH_3
	(n=1)	(n=2)		(n=1)	(n=2)	
1‴	93.4	94.5	93.3	94.5	94.5	CH
2‴	30.9	31.1	30.8	31.1	31.1	CH_2
3‴	74.9	75.0	74.8	75.0	75.0	CH
4‴	72.2	72.2	72.2	71.5	72.2	CH
5‴	65.4	65.1	65.4	66.6	65.0	CH
6‴	17.8	17.7	17.8	17.5	17.7	CH_3
3‴-OCH_3	55.9	56.2	56.0	56.4	56.1	CH_3

[a] Measured at 75 or 125 MHz in $CDCl_3$; chemical shifts in ppm from TMS.
[b] As determined by ^{13}C NMR DEPT experiments.

Table 4. Tabulation of ^{1}H NMR chemical shift assignments and coupling data for altromycins E~I in $CDCl_3$ (ppm from TMS).

Position	Altromycin E (**1**)			Altromycin F (**2**)			Altromycin G (**3**)			Altromycin H (**4**)			Altromycin I (**5**)		
	δ	m[a]	J, Hz	δ	m[a]	J, Hz	δ	m[a]	J, Hz	δ	m[a]	J, Hz	δ	m[a]	J, Hz
3	6.40	s	—	6.40	s	—	6.55	s	—	6.45	s	—	6.44	s	—
6	8.73	s	—	8.73	s	—	8.72	s	—	7.54	s	—	7.54	s	—
8	7.85	d	8.0	7.84	d	7.9	7.87	d	7.8	7.69	d	7.9	7.68	d	7.8
9	7.95	d	8.0	7.97	d	7.9	7.97	d	7.8	7.78	d	7.9	7.84	d	7.8
11-OH	13.20	s	—	13.20	s	—	13.15	s	—	13.38	s	—	13.38	s	—
13	5.75	br s		5.75	br s		—	—	—	—	—	—	—	—	—
15	1.91	s	—	1.91	s	—	1.93	s	—	1.90	s	—	1.91	s	—
16	3.32	q	5.5	3.34	q	5.5	3.37	q	5.3	3.36	q	5.5	3.36	q	5.4
17	1.32	d	5.5	1.31	d	5.5	1.28	d	5.3	1.31	d	5.5	1.32	d	5.4
19	3.74	s	—	3.72	s	—	3.72	s	—	—	—	—	—	—	—
2′	4.46	dq	7.1, 1.7	4.45	dq	7.2, 1.7	4.33	dq	7.3, 1.5	—	—	—	—	—	—
3′	4.01	dd	3.1, 1.7	4.01	dd	2.9, 1.7	3.97	dd	3.2, 1.5	—	—	—	—	—	—
4′	3.43	dd	9.0, 3.1	3.43	dd	9.1, 2.9	3.61	dd	8.9, 3.2	—	—	—	—	—	—
5′	4.49	dd	9.4, 9.0	4.49	dd	9.5, 9.1	4.69	dd	8.9, 8.8	—	—	—	—	—	—
6′	4.18	dd	9.4, 1.5	4.17	dd	9.5, 1.2	4.58	d	8.8	—	—	—	—	—	—
2′-CH_3	1.61	d	7.1	1.61	d	7.2	1.74	d	7.3	—	—	—	—	—	—
4′-OCH_3	3.52	s	—	3.52	s	—	3.62	s	—	—	—	—	—	—	—
2″	4.46	dq	7.1, 6.2	4.46	dq	6.9, 5.7	4.48	dq	7.0, 6.5	4.37	dq	7.0, 5.2	4.31	dq	6.8, 5.8
3″	3.80	d	6.2	3.77	d	5.7	3.77	d	6.5	3.87	d	5.2	3.73	d	5.8
5a″	2.40	d	14.2	2.49	d	14.3	2.06	dd	13.5, 2.0	2.40	dd	14.0, 3.5	2.46	d	14.5
5b″	1.22	dd	14.2, 9.5	1.24	dd	14.3, 10.9	1.51	dd	13.5, 11.2	1.49	dd	14.0, 10.0	1.19	dd	14.5, 10.7
6″	5.49	d	9.5	5.54	d	10.9	5.60	dd	11.2, 2.0	5.40	dd	10.0, 3.5	5.46	d	10.7
2″-CH_3	1.58	d	7.1	1.68	d	6.9	1.58	d	7.0	1.48	d	7.0	1.66	d	6.8
4″-CH_3	1.22	s	—	1.05	s	—	1.27	s	—	1.29	s	—	1.01	s	—
4″-$N(CH_3)_n$[b]	2.47	s	—	2.47	s	—	—	—	—	2.51	s	—	2.44	s	—
1‴	4.88	d	4.3	4.80	d	4.7	4.91	d	4.3	4.77	dd	3.7, 2.6	4.77	d	4.6
2a‴	2.31	dd	15.1, 3.5	2.32	dd	15.0, 3.2	2.34	dd	15.1, 3.2	2.30	ddd	14.8, 4.8, 2.6	2.30	dd	14.8, 3.2
2b‴	1.78	ddd	15.1, 4.3, 3.2	1.78	ddd	15.0, 4.7, 3.2	1.78	ddd	15.1, 4.3, 3.2	1.76	ddd	14.8, 3.7, 3.5	1.76	ddd	14.8, 4.6, 3.2
3‴	3.67	ddd	3.5, 3.2, 3.2	3.63	ddd	3.6, 3.2, 3.2	3.68	ddd	3.4, 3.2, 3.2	3.61	ddd	4.8, 3.5, 3.1	3.61	ddd	3.6, 3.2, 3.2
4‴	3.30	dd	9.4, 3.2	3.27	dd	9.7, 3.6	3.30	dd	9.5, 3.4	3.24	dd	8.4, 3.1	3.24	dd	9.6, 3.6
5‴	4.05	dq	9.4, 6.3	4.10	dq	9.7, 6.5	4.05	dq	9.5, 6.3	4.08	dq	8.4, 6.5	4.08	dq	9.6, 6.1
6‴	1.28	d	6.3	1.27	d	6.5	1.29	d	6.3	1.24	d	6.5	1.24	d	6.1
3‴-OCH_3	3.48	s	—	3.42	s	—	3.48	s	—	3.39	s	—	3.39	s	—

[a] m: multiplicity.
[b] n: (**1**), 1; (**2**), 2; (**3**), 0; (**4**), 1; (**5**), 2.

in $CHCl_3$-MeOH (95:5) and the altromycin-type compounds were pooled based on general polarity. The altromycins were then separated from impurities and each other by using both preparative and semipreparative countercurrent chromatography instruments with ratio variations of the solvent system MeOH-50 mM NH_4OAc (pH 5.0)-$CHCl_3$-CCl_4. The main product of these fermentations was altromycin B. From a total of 515 liters of fermentation broth, 4.2 g of altromycin B was isolated. Minors that differed spectroscopically from altromycins A, C and D were also present. Altromycins E, F, G, H and I were isolated in 4.4, 7.6, 4.7, 79 and 97 mg amounts respectively. The newly isolated orange-red altromycin minors were characterized mainly by UV, NMR and mass spectral and optical rotation data as shown in Tables 1~5.

Although altromycins E and F exhibited identical parent ions in FAB-MS as altromycins C and D respectively (Table 1), other spectroscopic data for these compounds differ significantly from C and D.[2] The UV data for altromycin G is essentially identical to that of altromycins C and D. However, a major change is seen in the base shifted adsorption spectra of both E and F in comparison to G (Table 2). This indicates a structural change about the anthraquinone-γ-pyrone nucleus. The ^{13}C NMR data of altromycins E and F has no quaternary carbon signal at ~80 ppm as found for C-13 in altromycin G, and a new methine signal appears at ~48 ppm (Table 3). Two dimensional ^1H NMR studies show that the proton on the 48 ppm methine (δ 5.75, broad singlet) shows both COSY and NOE exchange to the 6'-H pseudoanomeric proton on the C-glycosyl moiety attached to C-13 (Table 4). In addition, HMBC couplings were seen between both C-5 and C-6 of the B ring and the C-13 methine proton. These data indicate that the hydroxyl functionality has been lost on the C-13 position of altromycins E and F as compared to altromycins A~D and G.

Analysis of FAB-MS DCl-D_2O exchange experiments on altromycin G indicated that it had one more exchangeable proton than altromycin A, and FAB-MS data aquired under normal conditions indicated one less CH_2. This observation, combined with analysis of UV and NMR data, lead to the formulation of altromycin G as a minor variant of the A~B structure type. It differs only by virtue of having a primary amine functionality at C-4'' of the vancosamine moiety instead of a mono- or dimethylamine. The NMR spectra of altromycins H and I are missing the entire set of signals due to the elaboration of the C-5 B-ring position of the anthraquinone-γ-pyrone nucleus and the C-5 carbon signal has moved from ~148 ppm to ~166 ppm. FAB-MS data are consistent with the 166 ppm signal being that of a phenolic hydroxyl group on C-5. There are no data to support an absolute stereochemical assignment of the sugars in the altromycins. However, relative stereochemical relationships based on NOE and coupling constant data are shown, except at C-13, which remains undefined.

Table 5. Optical rotation data for altromycins E~I in methanol.

Altromycin	$[\alpha]_D^{25}$	Concentration c
E	+63°	0.05
F	+57°	0.09
G	+26°	0.06
H	+105°	0.10
I	+53°	0.02

The altromycins are novel compounds 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 remains unsubstituted. The major structural differences among the altromycins occur as additional novel elaborations from the C-5 B-ring position of the anthraquinone-γ-pyrone nucleus.

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