Antitumor Agents from the Bohemic Acid Complex.[1] 4. Structures of Rudolphomycin, Mimimycin, Collinemycin, and Alcindoromycin

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Abstract: The structures of four new anthracyclines (rudolphomycin (**11**), collinemycin (**12**), mimimycin (**13**), alcindoromycin (**14**)) have been determined by a combination of chemical degradations and spectral interpretation. The use of ^{13}C NMR spectroscopy has been extensive and assignments to the ^{13}C NMR spectra of these compounds have been made.

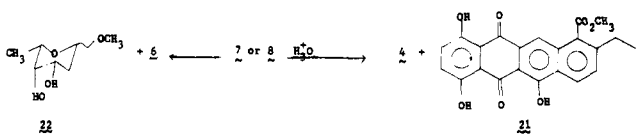
The marked clinical effectiveness of the antitumor antibiotics adriamycin and daunomycin (**1** and **2**) (Figure 1) has led to an intensive search for new members of this interesting class of compounds.[2] This search has resulted in the isolation and characterization of a number of new agents: carminomycin[3] (**3**), cinerubins A and B,[4] aclacinomycins A and B,[5] the rhodirubins,[6] baumycins,[7] nogalamycin,[8] and marcellomycin and musettamycin[9] from our own laboratories.

Recent work from these laboratories has shown that some of the ϵ-pyrromycinone-based anthracyclines as well as aclacinomycin A (**10**) (based on 1-deoxypyrromycinone) possess a mode of action which distinguishes them from the adriamycin class of anthracyclines.[10] In the preceding paper[11] of this series we have described the isolation of several new members of this unique class of agents. It is the purpose of this paper to give the structure determination of these compounds as well as to provide further details of the structure elucidations of musettamycin and marcellomycin.[9] The structures are illustrated in Table I.

Structures of Musettamycin (7) and Marcellomycin (8)

The structural assignments to musettamycin and marcellomycin which were made earlier were based on a number of lines of evidence as outlined below. Both compounds were reddish-orange solids having elemental formulas $C_{36}H_{45}NO_{14}$ and $C_{42}H_{55}NO_{17}$, respectively. Total acid hydrolysis of either **7** or **8** led to the isolation of ϵ-pyrromycinone (**4**) in addition to trace amounts of η-pyrromycinone (**21**) (Scheme I) which possessed identical properties with those reported by Brockmann and Lenk for compounds **4** and **21**.[12] Mild alcoholysis of **7** or **8** gave ϵ-pyrromycin (**6**), the NMR spectrum of which was identical with that reported for the partial hydrolysis product of cinerubin A (**9**).[4] Thus the aglycone as well as the first sugar for both these products was established. From the nonanthracycline portion of the methanolysis experiments the methyl glycoside of 2-deoxy-L-fucose (**22**) as a mixture of α and β anomers was obtained with the α anomer predominating. The ^1H NMR spectrum of musettamycin exhibited three signals in the anomeric region at δ 5.50, 5.24, and 5.00 as broad singlets which have been assigned to the C-1′, C-7, and C-1″ protons, respectively, by comparing the line positions for a series of related compounds (Table II). The small coupling constants indicated that the protons in question were equatorial rather than axial. Thus the structure of musettamycin was assigned as shown below for **7** (Figure 2). The methanolysis of marcellomycin (**8**) gave only **22** in addition to pyrromycin. An examination of the proton spectrum of **8** indicated the presence of an additional anomeric proton (α anomer) as well as other signals attributable to a third sugar in the molecule. Examination of the ^{13}C NMR spectrum of **8** in comparison with that of **7** confirmed the existence of a third sugar and indicated that it was probably a second 2-deoxyfucosyl residue.

Scheme I



22 **21**

At this juncture there was some question as to the point of attachment of the 2-deoxy-L-fucose residues. The ^1H NMR spectra of both **7** and **8** exhibited three exchangeable signals downfield for the phenolic protons at C-1, C-4, and C-6, thus ruling out attachment to these centers. It did not appear likely that the sugar residues could be attached to the tertiary C-9 hydroxyl, thus leaving only the C-4′ carbon as the point of attachment. A comparison of the ^{13}C NMR spectra of pyrromycin, musettamycin, and marcellomycin confirmed that these residues were linked through the C-4′ carbon (Figure 3). Note the shift of the C-4′ carbon on formation of the glycosidic linkage to the 2-deoxy-L-fucose in musettamycin (**7**). Similarly, a large shift for the C-4″ carbon was noted on going to marcellomycin from musettamycin. Details of the assignments are given in a later section on the ^{13}C NMR spectra of a number of these compounds.

The conformation shown for the D ring of the aglycone portion is based on the narrow coupling for the proton at C-7 as well as an examination of molecular models which indicates this to be the conformation in which the peri interactions between the sugar residue at C-7 and the C-6 hydroxyl and the C-10 carbomethoxy and C-11 proton are minimized.

Structure of Rudolphomycin (11)

The third anthracycline present in major amounts in the bohemic acid complex is rudolphomycin (**11**). It was isolated as an orange-red solid which analyzed for $C_{42}H_{52}N_2O_{16}\cdot{}^3/_2H_2O$.[13] High-resolution field desorption mass spectrometry confirmed this elemental formula exhibiting ions at *m/e* 841 (M + 1), 586, and 428 corresponding to the molecular ion and cleavages at the C-1″ and C-1′ anomeric carbons, respectively. The ultraviolet and visible spectra of rudolphomycin were superimposable on that of marcellomycin with the exception of a new absorption band at 280 nm which did not shift on addition of either dilute base or dilute acid (Figure 4). The IR spectrum of **11** showed carbonyl bands at 1735 and 1600 cm^{-1} and was similar in this respect to both **7** and **8** with the exception that the 1600-cm^{-1} band was relatively more intense than in either **7** or **8**.

As in the case of marcellomycin extensive hydrolysis of rudolphomycin gave ϵ-pyrromycinone while partial methanolysis yielded pyrromycin, thus establishing the structure of the ag-

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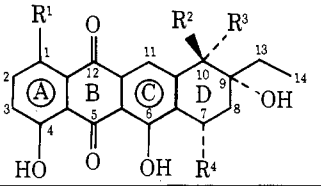
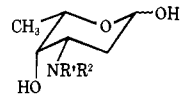
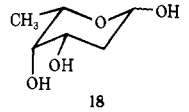
Table I. Structures



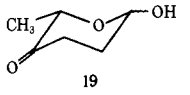
	R^1	R^2	R^3	R^4	
4	OH	CO_2CH_3	H	OH	ϵ-pyrromycinone
5	H	CO_2CH_3	H	α-Rh	aklavin
6	OH	CO_2CH_3	H	α-Rh	pyrromycin
7	OH	CO_2CH_3	H	α-Rh-4′-α-DF	musettamycin
8	OH	CO_2CH_3	H	α-Rh-4′-α-DF-4″-α-DF	marcellomycin
9	OH	CO_2CH_3	H	α-Rh-4′-α-DF-4″-α-Cin	cinerubin A
10	H	CO_2CH_3	H	α-Rh-4′-α-DF-4″-α-Cin	aclacinomycin A
11	OH	CO_2CH_3	H	α-Rh-4′-α-DF-4″-α-R	rudolphomycin
12	OH	H	CO_2CH_3	α-Rh-4′-α-DF	collinemycin
13	OH	H	CO_2CH_3	α-Rh-4′-α-DF-4″-α-DF	mimimycin
14	OH	CO_2CH_3	H	α-NDMRh-4′-α-DF-4″-α-DF	alcindoromycin



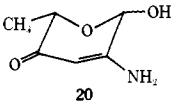
15, $R^1 = R^2 = H$ daunosamine (D)
16, $R^1 = R^2 = CH_3$ rhodosamine (Rh)
17, $R^1 = H; R^2 = CH_3$ *N*-demethylrhodosamine (NDMRh)



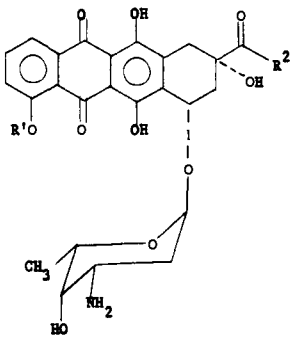
18 2-deoxy-L-fucose (DF)



19 cinerulose (Cin)

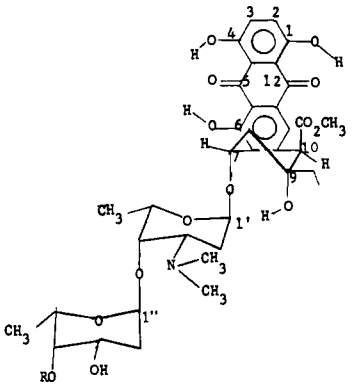


20 rednose (R)



1 $R_1 = CH_3$ $R_2 = CH_2OH$
2 $R_1 = R_2 = CH_3$
3 $R_1 = H$ $R_2 = CH_3$

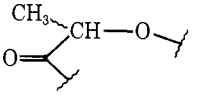
Figure 1. Structures of adriamycin (**1**), daunomycin (**2**), and carminomycin (**3**).



7 R = H
8 R = α-2-deoxyfucosyl

Figure 2. Structures of musettamycin (**7**) and marcellomycin (**8**).

lycone and the first sugar in rudolphomycin. This also proved that the 280-nm UV chromophore as well as the 1600-cm^{-1} IR band was located in the remaining sugar residues. The ^1H NMR spectrum of rudolphomycin exhibited signals in the anomeric region of the spectrum which integrated for five protons. Three of the resonances appeared at δ 5.53, 5.28, and 5.10 as broad singlets and could be assigned to the C-1′, C-7, and C-1″ protons (Table II). These resonances were almost superimposable on those of musettamycin and marcellomycin. In addition, two sharp singlets integrating for one proton each were observed at δ 5.26 and 5.32. The methyl region of the spectrum integrated for 12 protons, indicating that rudolphomycin contained 4 methyl groups. One of the methyl resonances was shifted downfield from the rest to δ 1.38 and appeared as a doublet. When the NMR spectrum of **11** was run in deuterated pyridine the protons on the oxygenated carbons were shifted apart, permitting a first-order analysis of the signals. In addition to those signals assignable to the aglycone and rhodosamine there were two methyl groups present, one of which was coupled to a proton appearing at δ 5.52 *which was not coupled to any other protons.* Furthermore, this methyl group appeared farthest downfield of the methyl signals. This suggested the following fragment in which the low-field position of the methyl group was accommodated.



In view of the structures of marcellomycin and musettamycin we suspected that the second sugar residue in rudolphomycin might also be 2-deoxy-L-fucose. The ^1H NMR

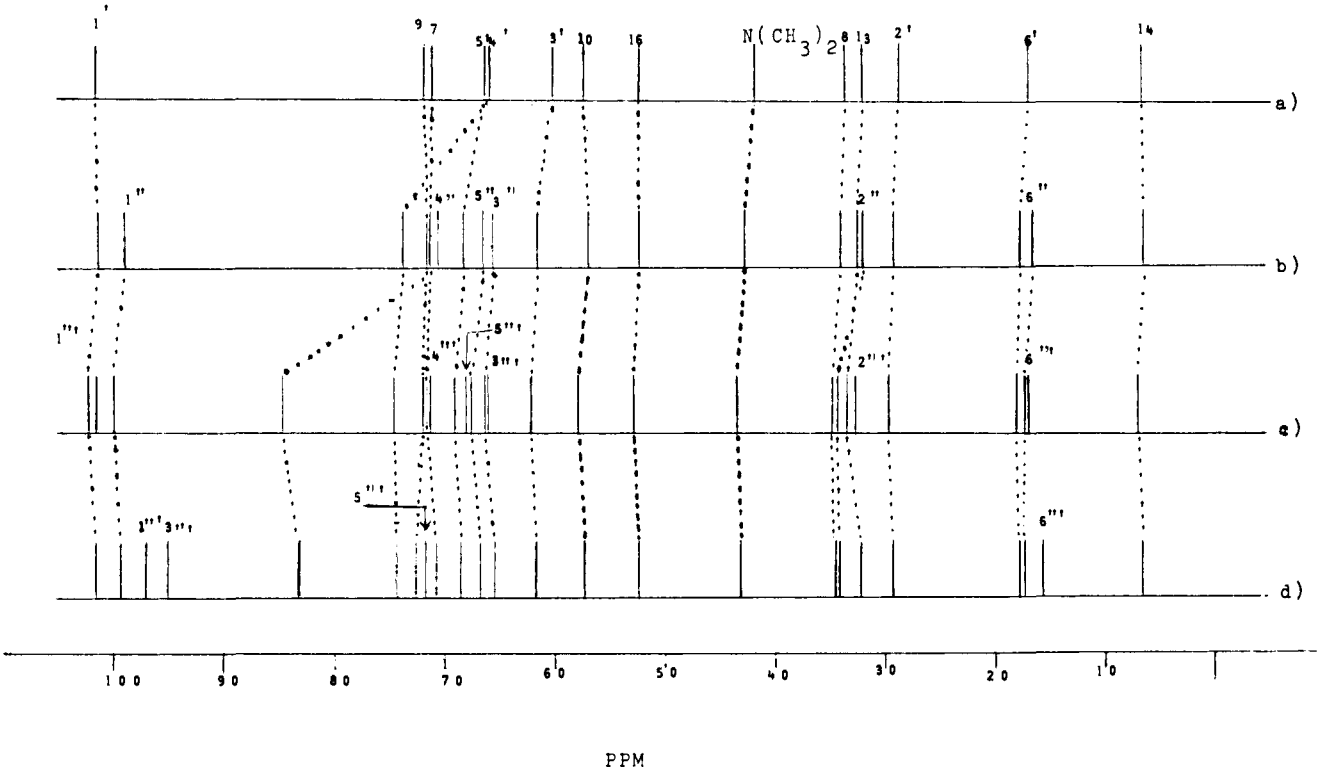


Figure 3. ^{13}C NMR line positions for the aliphatic portions of (a) pyrromycin (**6**), (b) musettamycin (**7**), (c) marcellomycin (**8**), (d) rudolphomycin (**11**). Lines given relative to internal Me_4Si.

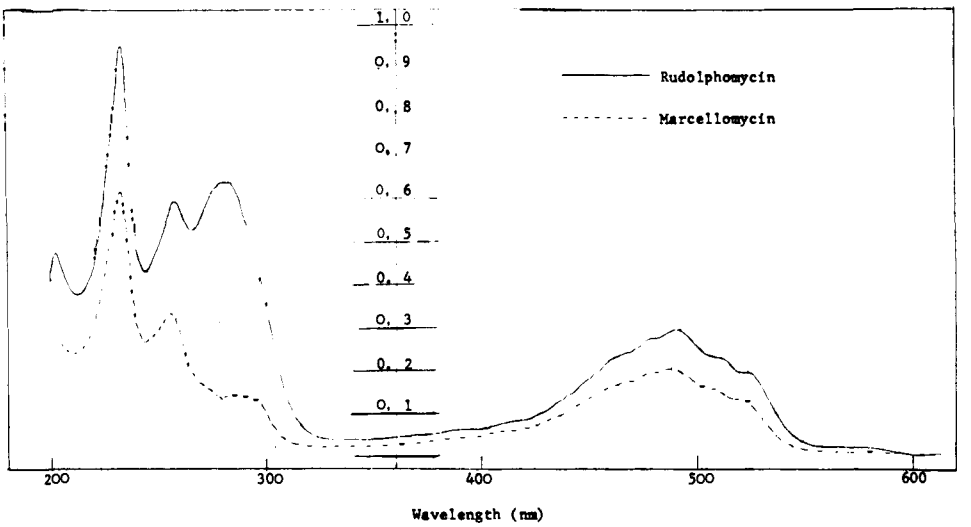
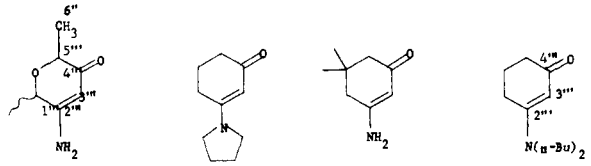


Figure 4. Comparative UV and visible spectra of marcellomycin and rudolphomycin.

Table II. ^1H NMR Chemical Shifts of Selected Protons

compd	C-1′H	C-7H	C-1″H	C-1‴H	C-3‴H	C-10H	CO_2CH_3
4		5.38				4.12	3.72
6	5.54	5.32				4.12	3.70
7	5.50	5.24	5.00			4.10	3.68
8	5.52	5.32	5.05	4.90		4.12	3.69
9	5.53	5.28	5.05	5.05		4.12	3.70
11	5.53	5.28	5.10	5.26	5.32	4.14	3.72
12	5.50	5.27	5.02			4.00	3.88
13	5.50	5.28	5.00	5.00		4.00	3.88
14	5.49	5.29	4.96	4.96		4.14	3.73
29			4.75	5.28	5.37		

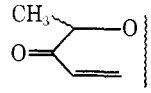
spectrum of **11** (both in $CDCl_3$ and C_5D_5N) supported this view, as did a comparison of the ^{13}C NMR spectrum of rudolphomycin (**11**) with those of **7** and **8**. The ^{13}C NMR spectrum of **11** was similar to that of musettamycin (**7**) with the exception that the C-4″ carbon was shifted to 83.1 ppm (vs. 84.6 observed for marcellomycin). While **11** gave signals for



	25	26	27	28
ir ν c=o	1600 cm^{-1}	1598 cm^{-1}	-	-
uv max	280 nm	-	287 nm	-
^{13}C nmr	-	-	-	-
C-2‴	157.8 ppm	-	-	164.7 ppm
C-3‴	95.1 ppm	-	-	98.2 ppm
C-4‴	194.1 ppm	-	-	196.2 ppm

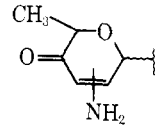
Figure 5. Structure of rednosyl residue **25** in rudolphomycin.

the third anomeric carbon (97.1 ppm) and the C-5‴ and C-6‴ carbons (at 70.7 and 15.7 ppm, respectively), no other aliphatic-type carbons could be seen. An examination of the downfield portion of the spectrum showed signals at 95.1, 157.8, and 194.1 ppm. The signal at 194.1 ppm could only be for the carbonyl carbon of an α,β-unsaturated ketone which would account for the three missing carbons of the hexose. This joined with the earlier derived fragment gave the partial structure shown in **23**.



23

Addition of an anomeric carbon and the extra nitrogen atom revealed by the elemental analysis and field desorption mass spectrum suggested the structure **24**.

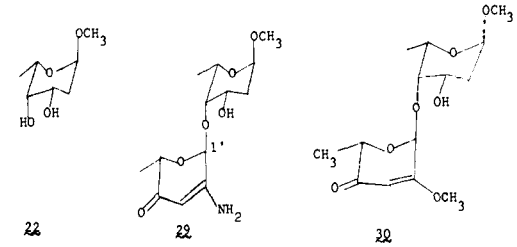


24

Placement of the amino function at C-2‴ was fully consistent with the physical data and so structure **25** is proposed for the third carbohydrate residue in rudolphomycin. Upon searching the literature we found that this probably represents the first example of a 2-amino-2,3,6-trideoxyhex-2-enopyranos-4-ulose, although the synthesis of a closely related compound was reported by Meyer zu Reckendorf.[14] In view of the origin of this sugar we propose the trivial name rednose for **25**.

While there are no known carbohydrates containing the β-enamino ketone function in the literature, there are a number of β-enaminones which may serve as models (Figure 5). The IR absorption of the carbonyl function in **26** has been reported as 1598 cm^{-1} compared to that of rednose at ~1600 cm^{-1}.[15] As a model for the UV absorption of rednose **25** one may use the β-enaminone **27** the UV maximum of which has been reported to occur at 287 nm.[16] The failure of **25** to exhibit a shift on addition of acid is probably due to the fact that β-enaminones only exhibit a shift when the UV is run in 0.1 N acid solution.[17] Finally the ^{13}C NMR of compound **28** has been reported.[18] Comparison of the carbon resonances of **28** with those assigned to **25** shows a good correspondence, the small shift differences seen being ascribable to the substitution of the nitrogen atom in **28**.

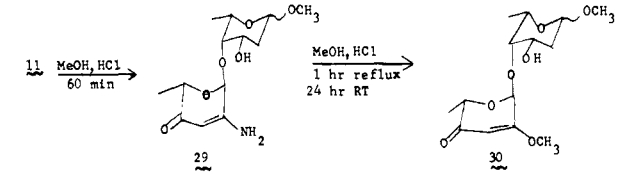
We next attempted to confirm the proposed structure by examining the nonanthracycline products resulting from the methanolysis of rudolphomycin. Attempts to detect the methyl glycosides of 2-deoxy-L-fucose in the methanolysis mixture failed completely and led to a more careful examination of the



	22	29	30
OMe	3.36 s	3.35	3.34
C-1H	4.80 bs	4.79	
C-2H	1.60-2.10 m	1.6-2.1	1.5-2.0
C-3H	4.0 ddd	4.04 ddd	~ 4.0
C-4H	3.66	3.94 bs	~ 3.76
C-5H	3.93 dq	3.98 d	3.92 dq
C-6H$_3$	1.30 d	1.30	1.28
C-1'H	-	5.57 s	5.41
C-3'	-	5.44 s	4.86
C-5'H	-	4.65 q	4.85
C-6'H$_3$	-	1.45	1.45
OCH$_3$	-	-	3.80
NH$_2$	-	6.36 bs	-

Figure 6. ^{1}H NMR spectra of 2-deoxy-L-fucose and disaccharides from rudolphomycin.

Scheme II



reaction. A sample of **11** was dissolved in methanol-methylene chloride and methanolic hydrogen chloride added (Scheme II). The reaction was followed by monitoring the disappearance of rudolphomycin (**11**) using high-pressure liquid chromatography.[11] As the amount of **11** decreased the levels of pyrromycin increased as well as two new peaks (in ~7:3 ratio) which eluted at greater retention volumes. When all of the rudolphomycin disappeared the reaction mixture was worked up to yield pyrromycin and two slower moving components. These were purified by semipreparative high-pressure liquid chromatography to yield **29a** and **29b**, respectively, the α and β anomers of the disaccharide derived from the terminal sugars of rudolphomycin. If the methanolysis reaction was allowed to proceed for 24 h (rather than ~1 h), compounds **29a** and **29b** disappeared only to be replaced by compound **30** (a mixture of α and β anomers of the disaccharide derived from methanolysis of the 2‴-amino function in **29**). The structures of **29** and **30** follow from their spectra as outlined below. The great stability of the anomeric linkage of the rednosyl residue with the 4 oxygen of the 2-deoxy-L-fucose explains the failure to observe **22** on methanolysis of **11**.

As in the parent compound **11**, the disaccharide **29** exhibited a maximum in the UV at 280 nm. In contrast, the maximum was shifted to 250 nm in compound **30**, which would be expected on replacement of an amino function by an alkoxy function in an α,β-unsaturated ketone.[19] Compound **29** was silylated using TriSil and the mass spectrum of the resulting silyl ether measured. A molecular ion at *m/e* 359 was observed and the fragmentation pattern was consistent with the proposed structure. The ^{1}H NMR was measured in both $CDCl_3$ and C_5D_5N and using spin decoupling techniques it was possible to assign the resonances for the 2-deoxy-L-fucose protons. Comparison of the spectrum of **29** with that of 2-deoxy-L-fucose α-methyl glycoside gave almost superimposable spectra

Table III. ^{13}C NMR Chemical Shifts[a]

carbon no.	**39**[b]	**5**[c]	**6**[c]	**7**[c]	**12**[f]	**8**[d]	**13**[c]	**14**[e]	**11**[e]	**10**[c]	**29**[c]	**30**[c]	ΔOH → OR[g]
1	119.5	120.0	157.9	157.4	157.7	158.7	157.6	157.1	158.5	120.0			
2	137.7	137.1	129.6	129.5	129.9	130.5	129.9	129.5	130.1	137.1			
3	125.0	124.6	130.1	129.9	130.0	130.8	129.5	131.8	129.6	124.6			
4	162.1	162.3	158.5	158.1	158.2	159.3	158.2	157.6	159.0	162.3			
6	156.6	161.9	162.4	161.9	162.2	163.2	161.4	161.8	162.3	161.9			
11	156.7	120.8	120.5	120.0	120.5	121.0	120.8	119.5	120.4	120.7			
5	190.1	192.4	190.6	189.8	190.4	191.6	190.2	189.3	190.6	192.4			
12	185.9	181.0	185.6	184.9	185.7	186.7	185.5	184.6	185.6	181.0			
4a	115.8	115.6	112.5	111.7	112.5	113.1	112.4	111.8	112.5	115.6			
5a	110.9	114.4	114.9	114.0	114.3	115.5	114.1	114.0	114.8	114.4			
11a	111.0	131.2	131.7	131.1	132.3	132.5	132.3	131.2	132.8	131.2			
6a	134.7	132.7	132.9	132.2	132.2	133.5	131.1	129.4	131.6	132.6			
10a	132.9	142.5	142.8	142.1	143.5	143.5	142.6	141.9	142.6	142.4			
12a	139.3	133.3	112.6	111.9	112.4	113.3	112.2	111.6	112.4	133.3			
7	61.2	71.0	71.1	71.2	71.4	71.3	70.9	70.5	70.7	70.5			+10.0
8	35.0	33.7	34.2	34.0	36.8	34.9	33.3	33.9	34.3	33.7			
9	71.5	71.7	71.8	71.4	72.0	71.7	71.9	70.8	72.6	71.6			
10	51.8	57.1	57.4	56.9	56.4	57.9	55.8	56.8	57.3	57.1			
13	32.7	32.1	32.3	32.5	33.1	33.5	29.6	32.6	32.3	32.1			
14	7.1	6.7	6.7	6.6	7.7	7.1	8.0	6.6	6.7	6.7			
15	171.3	171.0	171.2	171.0	171.4	172.0	171.2	170.5	171.2	171.1			
16	52.6	52.4	52.3	52.4	52.2	52.9	52.3	52.2	52.4	52.4			
1′		101.3	101.5	101.3	100.6	102.4	100.6	101.4	101.6	101.5			
2′		28.8	28.8	29.2	29.3	29.7	29.1	31.5	29.3	29.2			0.5
3′		59.5	60.1	61.5	61.8	62.2	61.3	54.4	61.7	61.4			+1.8
4′		65.9	66.4	73.6	74.1	74.6	73.9	76.8	74.3	73.9			+7.9
5′		66.4	66.9	68.2	68.4	69.0	69.1	67.7	68.5	68.3			+1.9
6′		17.0	17.0	17.8	18.0	18.2	17.9	17.4	17.9	17.8			+0.9
$N(CH_3)_2$		41.9	42.0	42.7	43.3	43.5	43.0	33.0	43.2	43.2			+1.1
1″				98.9	99.4	100.0	100.5	100.1	99.4	100.0	98.9	99.8	
2″				32.0	32.9	34.4	34.2	33.8	34.1	33.7	34.0	34.0	+1.6
3″				65.5	65.6	66.2	65.5	64.9	65.5	65.3	65.3	65.6	
4″				70.5	70.3	84.6	83.7	81.0	83.1	82.9	82.6	84.4	+13.1
5″				66.4	66.6	67.5	67.1	67.2	66.8	66.6	65.7	65.7	
6″				16.6	17.1	17.4	17.0	17.1	17.4	16.9	17.5	17.4	
1‴						101.6	99.2	100.1	97.1	99.2	97.2	97.7	
2‴						32.8	32.9	31.9	158.5	27.6	158.4	176.6	
3‴						66.1	65.4	64.8	95.1	33.5	94.5	98.6	
4‴						71.9	70.9	70.8	194.0	209.7	193.7	188.4	
5‴						67.9	66.7	67.1	71.7	71.7	72.7	65.9	
6‴						17.1	16.8	16.9	15.7	14.8	15.7	16.8	

[a] In parts per million downfield from Me_4Si. [b] Me_2SO-d_6. [c] $CDCl_3$. [d] CD_2Cl_2. [e] $CDCl_3$-Me_2SO-d_6 (4:1). [f] CD_2Cl_2-Me_2SO-d_6 (4:1). [g] Average steric compression shifts on formation of glycosidic linkage at C-7,C-4′ of rhodosamine and C-4″ of 2-deoxy-L-fucose.

with the exception of the resonance for the C-4 proton, which was displaced to lower field in the disaccharide as would be expected (Figure 6). The ^{13}C NMR spectra of **29** and **30** also supported the assigned structure (Table III). It is readily apparent that **29** contains the same chromophore as in rudolphomycin, the signals for the carbons in **29** being virtually superimposable on those for the corresponding carbons in rudolphomycin (**11**). The methoxy derivative **30** exhibits much the same ^{13}C NMR for the 2-deoxy-L-fucose part of the molecule but the carbons in the terminal sugar portions exhibit shifts in keeping with the nature of the substitution which has been made.[20]

With the gross structure of the third sugar in rudolphomycin thus established, there remained the determination of the absolute configurations at C-1‴ and C-5‴. There were a number of lines of evidence bearing on this point including the ^1H NMR spectrum and optical rotatory dispersion curve for **29**, the biological fate of rednose in the fermentation, and a consideration of the expected conformational properties of the hex-2-enopyranos-4-ulose system. When one examines the high-resolution NMR spectrum of **29**, the signals for the protons at C-1 and C-3 appear as sharp singlets, there being no observable coupling. It has been shown[21] that pseudoaxial anomeric protons in hex-2-enopyranos-4-uloses couple to the allylic proton at C3 while equatorial anomeric protons do not. Consequently we conclude that in rudolphomycin and **29** the substituent at C1‴ is most likely pseudoaxial and that the C1‴ proton is pseudoequatorial. This would not be too surprising in view of the known preference for polar functions at an anomeric carbon to be axial (the anomeric effect).[22a] In addition, this effect would be reinforced by the "double bond-no bond resonance" interaction of the same polar function with the C2‴-C3‴ double bond.[23b] Finally, an axial configuration would minimize interactions between the 2‴-amino function and the alkoxy group which are eclipsed in pseudoequatorial configurations. It is known that the sign of the Cotton curve for the 350-nm n→π* R band in α,β-unsaturated ketones is determined by the chirality of the enone chromophore[23] and not by the configuration of functions attached to the ring. However, to the extent that such substituents influence the conformation of the ring containing the chromophore, they may affect the sign of the Cotton-effect curve. Consequently we have measured the ORD curve for **29** and have found a positive Cotton effect for the 350-nm n→π* transition (Figure 7). An examination of models for both possible geometrical isomers of the β-enaminone **29** indicates there are two conformational extremes for each isomer in which the glycosidic oxygen assumes the pseudoaxial conformation. For the trans

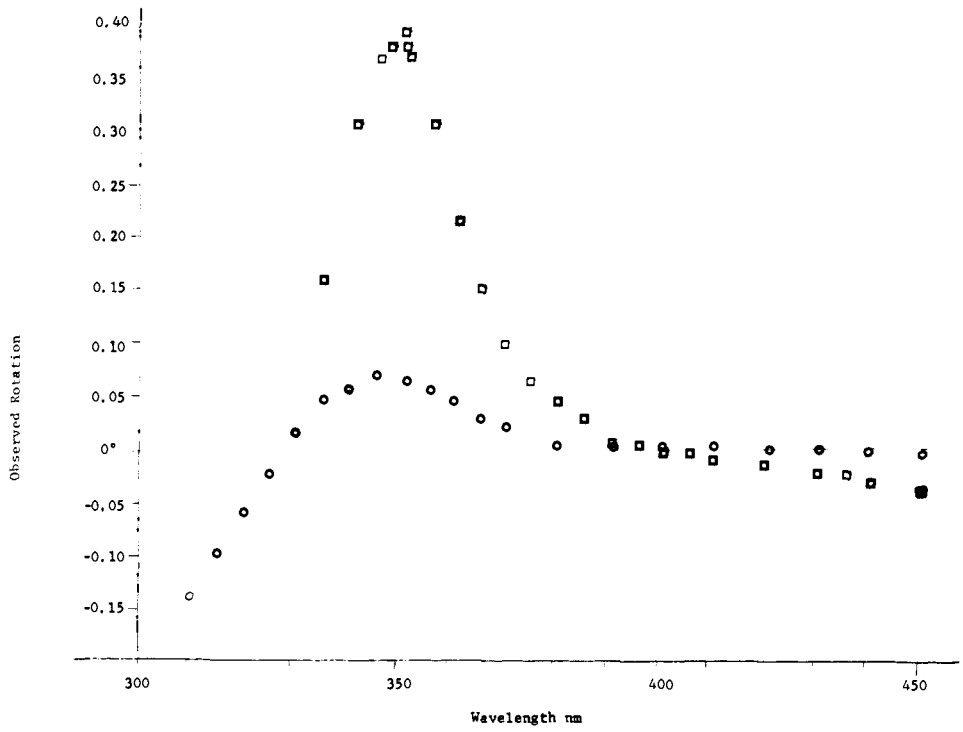


Figure 7. Optical rotatory dispersion of **29** at two different concentrations: □, 2.09×10^{-3} M; ○, 0.42×10^{-3} M in CH_2Cl_2.

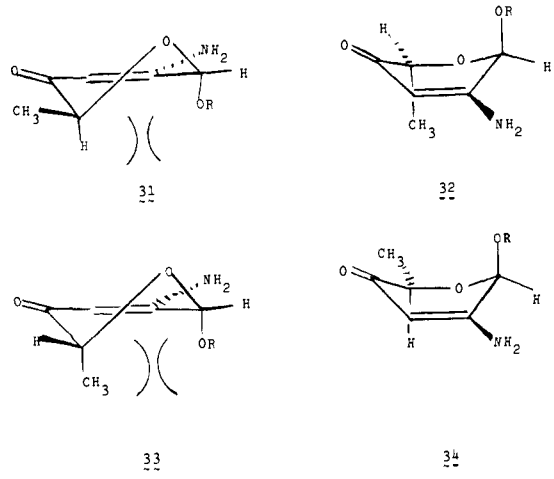


Figure 8. Conformations of cis and trans isomers of rednose.

isomer these may be represented by **31** and **32** in Figure 8. When one examines models of **31** and **32** it is evident that in conformer **31** there are two interactions which would destabilize this conformer relative to the boat conformer **32**: the eclipsing interaction of the C5 methyl group with the carbonyl oxygen and the syn-diaxial interaction of the anomeric oxygen substituent and the C5 proton. In the boat conformation the C-5 methyl group is subject to few interactions except those with the electron pair on oxygen or the π electrons of the double bond. Such interactions are expected to be minimal.[24] On this basis conformer **32** may be expected to be the major contributor to the conformational equilibrium of the trans isomer in solution. A similar analysis of the two conformational extremes for the cis isomer **33** and **34** shows that **34** should be the major contributor to the conformational equilibrium of this isomer. In this case the twist-boat conformer **33** has a severe syn-diaxial interaction between the methyl and alkoxy substituents. It is evident that the boat conformers **32** for the trans isomer and **34** for the cis isomer would be expected to predominate in solution and would *govern the chirality of the α,β-unsaturated enone.* It is also evident that **32** and **34,** irrespective of the different configurations at C-5, have identical chirality at C-1 and in the enone chromophore. If rednose has the absolute configuration about C-1 illustrated for **32** or **34** in Figure 8, then one would predict a positive Cotton-effect curve for **29,** which is what has been observed (Figure 7).

The one point of the structure remaining to be determined is the configuration at C-5‴ in the rednose residue. As yet we have been unable to resolve this point chemically; however, a clue to this question may exist in the biosynthetic origin of rudolphomycin and marcellomycin. During the course of the fermentation of the antibiotic complex, samples were periodically removed and analyzed by LC for the content of particular antibiotic components. It was observed that rudolphomycin was produced early in the fermentation. As the fermentation proceeded, the rudolphomycin content decreased while the marcellomycin content increased. It is possible that the terminal 2-deoxy-L-fucosyl residue in marcellomycin is biochemically derived from the rednosyl residue in rudolphomycin. If this is so and one can assume that the configuration at C-5‴ is not altered in the course of biotransformation, then the complete structure for rudolphomycin is as illustrated in Figure 9.

Structures of Mimimycin (13) and Collinemycin (12)

During the course of the isolation[9,11] of musettamycin (**7**), marcellomycin (**8**), and rudolphomycin (**11**) it was determined that a number of minor components were also present in the complex. Large-scale isolation of marcellomycin permitted accumulations of these minor components in sufficient amounts for structure determination. One of these components, mimimycin (**13**) (Table I), was eluted just after the main marcellomycin peak in the chromatography and was present at levels ~10% of that of marcellomycin (as estimated by LC).

The elemental formula of **13** was $C_{42}H_{55}NO_{17}$, identical with that of marcellomycin (**8**). In addition, the UV spectrum was identical and the IR spectrum very similar to that of **8**. Examination of the proton spectrum of **13** revealed that it was

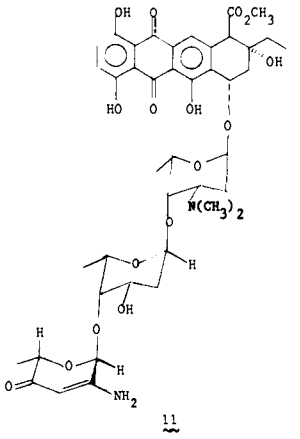


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Figure 9. Structure of rudolphomycin.

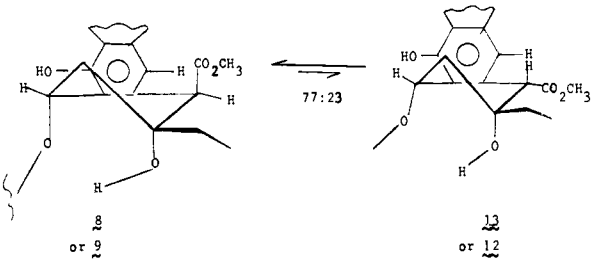
almost identical with that of **8** with the exception of the resonances for the C-10 proton and the carbomethoxy methyl group. The chemical shifts for the proton resonances of a number of compounds isolated in this work or otherwise available to us are listed in Table II. As is readily seen, the C-10 proton was shifted to higher field in **13** and the carbomethoxyl group's methyl shifted to lower field. The ^{13}C NMR spectrum of mimimycin (**13**) was almost identical with that of marcellomycin (see Table III) (**8**) with the exception of the resonances for C-10, C-13, C-14, and C-8, which showed slight shifts from the positions seen for **8**. As a result we felt that mimimycin was probably an epimer of marcellomycin at either C-9 or C-10. A test of the hypothesis that the compounds were epimeric at C-10 was readily carried out. Treatment of marcellomycin in methylene chloride solution with 1,5-diazabicyclo[4.3.0]-non-5-ene (DBN) led to an appearance of a new peak in the chromatogram having a retention volume identical with that of mimimycin. Similarly, treatment of mimimycin with DBN gave marcellomycin. The equilibrium ratio of marcellomycin to mimimycin was 77:23 (as estimated from peak heights).[25] The NMR results were readily interpretable in terms of the most stable conformers of **8** and **13** (Figure 10). In **8** the carbomethoxy projects above the plane of the C ring and thus the methyl resonance is shielded while in **13** the C-10 proton is shielded whereas the methyl group is deshielded relative to the situation in **8**.

Another minor component of the mixture was found to be closely associated with musettamycin (**7**). Upon isolation, collinemycin (**12**) (Table I) was found to have the same elemental formula and ultraviolet spectrum and a very similar infrared spectrum. Inspection of the ^1H NMR of **12** showed that, as in mimimycin, the methoxy resonance was shifted to lower field while the C-10 proton was shifted to higher field. As in the case of **13**, treatment of **12** with DBN promoted rapid equilibration to a mixture of musettamycin (**7**) and collinemycin (**12**) in a 77:23 ratio (estimated), thus establishing the structure of collinemycin as the C-10 epimer of musettamycin.

We cannot be certain if collinemycin and mimimycin are direct products of fermentation or artifacts of the extraction procedure. Analysis of crude extracts indicates the presence of these components at levels ~10% that of their C-10 epimers. These levels are less than the equilibrium concentrations. It is also conceivable that they are produced via epimerization of marcellomycin and musettamycin following the biosynthesis. In any event the free-energy difference between the epimers is small, being of the order of 0.7-0.8 kcal.

Structure of Alcindoromycin (14)

The third minor component of the bohemic acid complex which we have isolated is alcindoromycin (**14**) (Table I).



8 or 9 13 or 12

Figure 10. Equilibration of C10 proton.

Compound **14** was obtained as a red solid having the elemental composition $C_{41}H_{53}NO_{17}$. The infrared and ultraviolet spectra were similar to those for marcellomycin. The ^1H and ^{13}C NMR spectra, however, exhibited some differences.

The ^1H NMR spectrum of **14** was similar to that of marcellomycin with the exception of the *N*-methyl signal, which integrated for only three protons and was shifted downfield, δ 2.37 vs. 2.19 in marcellomycin. A comparison of the ^{13}C NMR spectrum of marcellomycin (**8**) with that of alcindoromycin (**14**) (Table III) showed that the two spectra were almost superimposable except for the carbon resonances corresponding to the rhodosamine portion of the spectrum. These resonances have been assigned to *N*-monodemethylrhodosamine (or *N*-monomethyldaunosamine). The isolation of *N*-monodemethylaclacinomycin A containing this sugar was recently reported by Oki et al.,[26] although no details of the structural assignment were given. Our assignment is based primarily on the ^{13}C NMR spectrum and is discussed in the following section.

Thus alcindoromycin is assigned the structure of *N*-monodemethylmarcellomycin.

^{13}C NMR Spectra of Anthracyclines

The structural assignments to the anthracyclines reported in this work have been made to greater or lesser extent upon analyses of their ^{13}C NMR spectra. These are given in Table III along with the spectra of a number of reference compounds, ϵ-rhodomycinone (**39**), aklavin (**5**), and aclacinomycin A (**10**).[27] The assignment of the carbons of the aglycones has been made using comparison of the spectra with known compounds, off-resonance decoupling, and theoretical models.[8,28] The assignments to the aglycone carbons may be considered reasonably certain; however, where there are resonances which occur very close to one another (e.g., resonances for carbons 2 and 3 in compounds **6, 7, 8, 11, 12, 13,** and **14**), these may conceivably be reversed. The assignments to the carbohydrate carbons were less straightforward.[29]

The observed average compression shifts for O-glycosidation are also recorded in the table.[30] These were derived from comparisons between spectra. There appears to be quite a difference between the effects of substitution of the 4-hydroxyl function of rhodosamine and 2-deoxy-L-fucose residues. The latter produced the expected large shift at the point of substitution and a moderate shift of the syn-axial carbon 2 with little or no shift of the other carbons. Substitution of rhodosamine gave a large shift at C-4 with moderate shifts at C-3 and C-5 and only a minimal shift at C-2. Possibly this is due to a flattening of the ring in the C-3–C-5 region so as to relieve steric interactions between the C-4 substituent and the bulky *N,N*-dimethylamino function at C-3.

It is also notable that a comparison of the C-5 and C-6 resonances of rednose in rudolphomycin (**11**) and of cinerulose in aclacinomycin A (**10**) confirmed the inferences drawn earlier from the ^1H NMR concerning the environment of these carbons.

The assignment to the amino sugar of alcindoromycin (**14**) as being *N*-monomethyldaunosamine (or *N*-monodemethyl-

rhodosamine) was made by comparing the resonances for the carbons of this sugar with those of daunosamine[31] and rhodosamine and with models from the literature.[32] The positions of the carbon resonances agree closely with those predicted on the basis of the models. This, coupled with the biogenetic origin of **14**, the ^{1}H NMR spectrum, and the literature precedent,[26] establishes the structure.

Interestingly the only consistent indication of the C-10 epimers collinemycin (**12**) and mimimycin (**13**) seen in the ^{13}C NMR spectra was the shift of the C-14 carbon to lower field. A small downfield shift of C-10 was also seen.

Finally the major problem in the case of marcellomycin was the assignment of the *CH*O carbon resonances, of which there are 11, not including the anomeric carbons, appearing between 66 and 84 ppm. A number of these could be readily assigned using shift data and spectral comparisons; however, several resonances were ambiguous, especially C-3″, C-5″, C-3‴, and C-5‴. These were assigned using the partially relaxed Fourier transform technique of Allerhand and Doddrell.[33] The resonances exhibiting the longer T_1's were assigned to the terminal 2-deoxy-L-fucose. Support was lent to the other assignments by these results as well.

The disaccharides **29** and **30** served as good models for the terminal sugars in rudolphomycin as well as 2-deoxy-L-fucose in marcellomycin, mimimycin, and aclacinomycin. In these two compounds (**29** and **30**) it is notable that C3′ and C5′ for the 2-deoxy-L-fucose residue come at 65.3-65.7 ppm. Comparing the ^{13}C NMR spectra of pyrromycin and musettamycin one observes that on going from pyrromycin to musettamycin there is a shift of the C3′ carbon in rhodosamine of 1.5-1.9 ppm to lower field. It seems reasonable to assume that a similar shift for the C5′ carbon should also occur. Consequently we have assigned the resonance at 68.2 ppm to the C5′ carbon of the rhodosamine residue in musettamycin. When one compares the average shift of the C3 and C5 resonances of rhodosamine in the seven di- and trisaccharides they are 1.8 and 1.9 ppm, respectively. With the assignment of the signal at 68.2 ppm to C5′ of rhodosamine this leaves the signals at 65.5 and 66.4 ppm to be assigned to the C3″ and C5″ carbons of the 2-deoxy-L-fucose residue in musettamycin. It is notable that these assignments are perfectly compatible with the values for the same resonances in 2-deoxy-L-fucose found in the disaccharides **29** and **30**. The assignments to marcellomycin were made similarly with the signal at 69.0 ppm being assigned to C5′ and resonances at 66.2, 67.5, 66.1, and 67.9 ppm being assigned to C3″, C5″, C3‴, and C5‴, respectively. The latter assignments were confirmed using T_1 measurements. The resonances having the longer relaxation times were assigned to C3‴ and C5‴. In view of the fact that C3′ has a longer relaxation time than C5′ the carbon having the longer relaxation time in each set was assigned to C3″ and C3‴, respectively. The assignment of the higher field signal to the C3″ and C3‴ resonances is also in accord with the literature in the sugar series.[34] The relaxation times for the other sugar resonances, the assignments of which are more straightforward, were in agreement with the assignments, thus providing an internal check of the method.

Experimental Section

Starting Materials. The isolation and physical properties of aklavin (**5**), marcellomycin (**8**), musettamycin (**7**), aclacinomycin A (**10**), rudolphomycin (**11**), collinemycin (**12**), mimimycin (**13**), and alcindoromycin (**14**) have been described in detail elsewhere.[5,9,11,27] Aklavin (**5**) was generously donated by Dr. W. A. Remers and aclacinomycin A (**10**) was obtained from Sanraku-Ocean, Ltd. The field desorption mass spectrum was run by Dr. K. Rinehart, Jr., whose assistance is gratefully acknowledged. We thank Dr. R. D. Brown of these Laboratories for the mass spectrum of **29**.

Formation of 29 from Rudolphomycin (11). A solution of 420 mg (0.5 mmol) of **11** in 10 mL of 1% methanolic hydrogen chloride was allowed to stand at room temperature for 1 h. The disappearance of the rudolphomycin was monitored using analytical high-pressure liquid chromatography. A Waters μ-Porasil column was used (3.9 mm × 30 cm) and the mobile phase was methylene chloride-methanol-concentrated ammonium hydroxide (96:4:1) at a flow rate of ~1.0 mL/min. Detection was by ultraviolet absorption at 254 nm. As the reaction proceeded the peak corresponding to rudolphomycin (k' = 1.69) disappeared and three new peaks appeared at k' = 0.46, 3.54, and 4.23 which were assigned to pyrromycin (**6**), the β anomer of **29**, and the α anomer of **29**, respectively. After 1 h the reaction was essentially complete. The solution was evaporated at reduced pressure to a small volume (1-2 mL) at which time a precipitate formed which was removed by filtration. A total of 165 mg of pyrromycin hydrochloride was collected which was identified via comparison with an authentic sample (same NMR spectrum and LC retention volume). The mother liquors from the reaction were evaporated to dryness and were taken up in methylene chloride (25 mL). The solution was chromatographed on 20 g of Woelm activity 1 silica gel using first methylene chloride (100 mL), then 5% methanol-methylene chloride (100 mL), then 7% methanol-methylene chloride (100 mL), and finally 10% methanol-methylene chloride as the eluting solvent. Ten 40-mL fractions were taken and fractions 4-7 contained the desired disaccharide unit **29** as a mixture of anomers in ~7:3 ratio with the α anomer predominating. A total of 103 mg of the anomer mixture was obtained (73%). Separation of the two isomers was achieved using semipreparative high-pressure liquid chromatography on a Partisil 20 (20μ silica gel microparticulate packing) column (10 mm × 50 cm). A solution of 100 mg of the crude **29** in 1.5 mL of a methylene chloride-methanol-concentrated ammonia solvent system (96:4:1) was loaded onto the column and developed at a flow rate of 4 mL/min. Fractions (28 mL) were taken and analyzed by LC. Fractions 7-8 were combined to yield 28 mg of the pure α anomer. Fractions 9-10 gave 30 mg of an isomer mixture and fraction 11 gave 3 mg of the β anomer enriched to 90% of the mixture.

Compound **29a** (α anomer) was obtained as an oil: UV (MeOH) λ_{max} 280 nm (ε 15 100); IR (liquid film) 3300-3400, 1580, 1440, 1365, 1250, 1210, 1122, 1095, 1035, 982, 920 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 1.35 (d, 3 H, J = 7 Hz, CH_3), 1.55 (d, 3 H, J = 7 Hz, CH_3), 1.77-2.39 (m, 2 H, CH_2), 3.14 (s, 3 H, OCH_3) 3.84 (dq, 1 H, J_1 = 7, $J_2 \simeq$ 1 Hz, C5H), 4.06 (bs, 1 H, C4H), 4.33 (ddd, 1 H, J_1 = 9.5, J_2 = 7.5, J_3 = 3.5 Hz, C3H), 4.76 (bs, 1 H, C1H), 5.38 (q, 1 H, J = 7 Hz, C5′H), 5.54 (s, 1 H), 5.70 (s, 1 H); mass spectrum of Me_3Si derivative *m/e* 359.

Compound **29b** was obtained as an oil. The IR and UV spectra closely resemble those of **29a**: ^{1}H NMR ($CDCl_3$) δ 1.39 (d, J = 7 Hz, 3 H, CH_3), 1.45 (d, J = 7 Hz, 3 H, CH_3), 1.5-2.2 (m, 2 H, CH_2), 3.53 (s, 3 H, OCH_3), 3.62 (dq, 1 H, J_1 = 7, J_2 = 1 Hz, C5H), 3.81 (bs, 1 H, C4H), 3.7-3.8 (bm, 1 H, C3H), 4.40 (dd, 1 H, J_1 = 9.5, J_2 = 2.0 Hz, C1H), 4.63 (q, 1 H, J = 7.0 Hz, C5′H), 5.43 (s, 2 H, C1′H and C3′H), 6.0 (bs, 2 H, NH_2).

Formation of 30. A solution of 420 mg (0.5 mmol) of **11** in 5 mL of methanolic hydrogen chloride (1%) was refluxed for 15 min. At the end of this time a reddish orange precipitate was formed. The suspension was let cool and filtered to yield 150 mg (70%) of ε-pyrromycinone contaminated with small amounts of λ-pyrromycinone (as shown by TLC in two systems against authentic standards and ^{1}H NMR spectroscopy). The mother liquors were refluxed for an additional 1 h and let stand for ~16 h at ambient temperature. The solvent and excess hydrogen chloride were removed at reduced pressure. The residue was taken up in 1 mL of 10% methanolic methylene chloride and chromatographed on 10 g of Woelm silica gel (activity 1). The first fractions from the column gave an additional 25 mg of ε-pyrromycinone. Intermediate fractions containing noncolored UV-absorbing materials were combined to yield 84 mg of a mixture of compounds. These were subjected to semipreparative LC as in the previous example using a Partisil 20 column (10 mm × 50 cm). Fractions 1 and 2 were combined to yield 29 mg of **30** as a mixture of α and β anomers. Fractions 4-6 were combined to give 25 mg of **29** as a mixture of α and β anomers.

Compound **30** was obtained as an oil, UV (MeOH) λ_{max} 250 nm (ε 17 900). The ^{1}H NMR and ^{13}C NMR spectra of **30** are recorded in Figure 6 and Table III, respectively.

Epimerization of Mimimycin (13). To a solution of 1.9 mg of mimimycin in 0.2 mL of methylene chloride was added 1 μL of 1,5-diazobicyclo[4.3.0]non-5-ene (DBN). The solution was let stand at ambient temperature (~21 °C) for 24 h. The reaction course was

followed by removal of 1-μL aliquots and analysis of these aliquots using high-pressure liquid chromatography (LC) as described for the earlier experiments (methanolysis of rudolphomycin to give **29**). Rapid disappearance of the peak for mimimycin (**13**) with concomitant appearance of a peak having the same retention volume as marcellomycin (**8**) was observed. At equilibrium the ratio of **8** to **13** was 77:23 as estimated by peak integration.

A similar experiment using marcellomycin (**8**) and forming mimimycin (**13**) was carried out. The mimimycin thus formed was isolated by preparative LC[11] and shown to be identical in all respects with that occurring in the crude aminoglycoside mixture.

Epimerization of Collinemycin (12). Treatment of 0.5 mg of **12** in 0.5 mL of methylene chloride with 2 μL of DBN and allowing the solution to stand gave an equilibrium mixture of musettamycin (**7**) and collinemycin (**12**). Similarly musettamycin produced the same equilibrium mixture. At equilibrium the ratio of **7** to **12** was approximately 77:23.

References and Notes

(1) It is the practice in these laboratories to give trivial names to antibiotic complexes early in the isolation effort. In view of the colorful nature of the anthracyclines we have designated these for receipt of operatic names. This particular antibiotic complex has been named bohemic acid (after La Boheme) and is produced by an actinosporagium sp. strain C36145 (ATCC 31127). Individual components of the complex, when these are unknown, are given names based on the characters in the opera. A preliminary account of a portion of this work was presented at the 1978 Chemical Institute of Canada's Meeting held at Winnipeg, Manitoba, Canada. This study was supported, in part, by Contract 1-CM-77147 from the Division of Cancer Treatment, National Institute of Health, Education, and Welfare.

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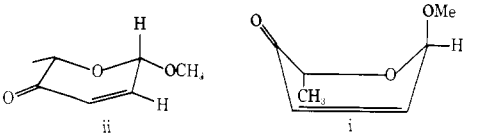

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