A NEW AMINOGLYCOSIDE ANTIBIOTIC COMPLEX—THE SELDOMYCINS

III. THE STRUCTURES OF SELDOMYCIN FACTORS 1 AND 2+

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(Received for publication August 12, 1976)

The structures of seldomycin factors 1 and 2 have been determined by consideration of chemical degradation and spectral properties. Factor 1, also known as XK-88-1, is shown to be 6-O-(2-amino-2-deoxy-α-D-xylopyranosyl) paromamine (**1**) and factor 2, also known as XK-88-2, is shown to be 4′-deoxy-neamine (**2**). Mass spectral evidence has been obtained that suggests the most probable structure for seldomycin factor 3, also known as XK-88-3, is 6′-amino-6′-deoxyseldomycin factor 1 (**12**).

The seldomycin factors are a group of aminoglycoside antibiotics isolated from the fermentation of a novel species of *Streptomycetes*, *S. hofunensis*. The mixture of antibiotics consists of four compounds previously designated XK-88-1, -2, -3, and -5 now generically named seldomycin factors 1, 2, 3 and 5. Previous papers in this series[1,2] have presented the fermentation, isolation and general characterization of these compounds. This paper will outline the evidence for the structures of seldomycin factors 1 and 2 and offer a suggestion for the probable structure of factor 3. The following paper in this series[3] presents the structural evidence for seldomycin factor 5, therapeutically the most significant.

Structure of Seldomycin Factor 1

The 100 MHz PMR spectrum of a D_2O solution of seldomycin factor 1 free base (**1**) (Fig. 1) indicated that this material is a pseudo-trisaccharide since two anomeric resonances are visible at low field. The anomeric coupling constants are both 4.0 Hz compatible with the α-D anomeric configuration generally found in other pseudo-trisaccharide aminoglycosides. Spin decoupling experiments revealed that both anomerics are affected by irradiation at 3.23 ppm. These relatively high field chemical shifts of H-2′ and H-2″ require that both are shielded by amine nitrogens and indicate that both sugars are 2-deoxy-2-amino derivatives.

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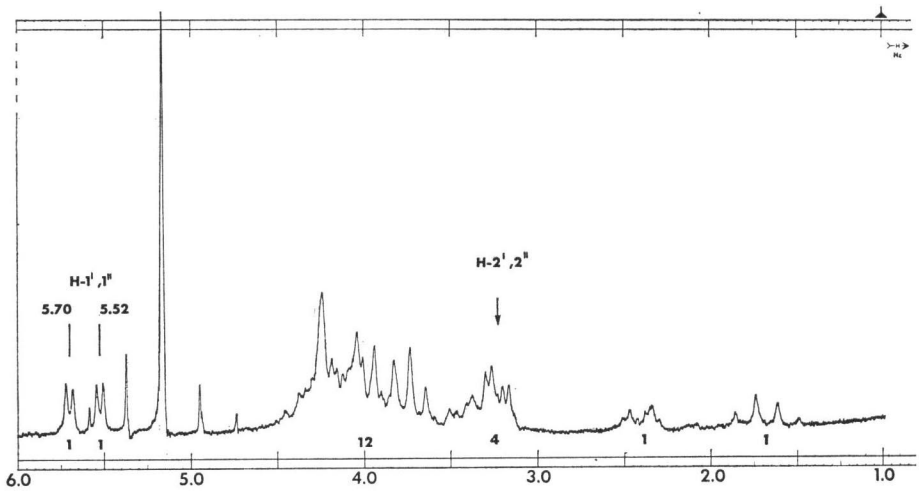
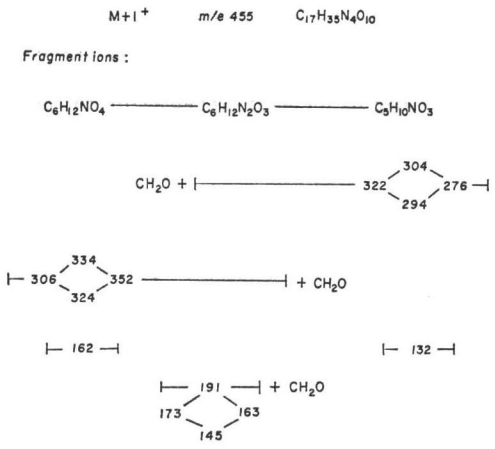
+ Also known as XK-88-1 and -2.

Fig. 1. 100 MHz PMR spectrum of seldomycin factor 1 free base (**1**) in D_2O solution.



The mass spectrum (Fig. 2) of seldomycin factor 1 free base gave a weak protonated molecular ion but sufficient for high resolution peak matching which gave a $C_{17}H_{35}N_4O_{10}$ formula. Consideration of fragment ions[4] allowed identification of the formulas of each component of the pseudo-trisaccharide. In particular, recognition of the characteristic tetrad of ions resulting from the sequential loss of H_2O and CO from the fragments arising from both possible pseudo-disaccharides permitted determination of the molecular formulas as shown in Fig. 2. Ions were also detected arising from each of the individual components at *m/e* 132, 162 and 191 indicating the presence of an aminohexose, diaminocyclitol and aminopentose (Fig. 2).

Fig. 2. Schematic representation of seldomycin factor 1 (**1**) mass spectrum.



The identity of the aminocyclitol was established when 2-deoxystreptamine (**3**) was shown to be present when seldomycin factor 1 was subjected to periodate oxidation followed by hydrolysis. This experiment also defines the compound as a 4,6-diglycoside as only then would 2-deoxystreptamine survive periodate oxidation.

Hydrolysis with 2 N HCl at 100°C gave a complex mixture of products which could be separated only after extensive ion-exchange chromatography. The identity of the aminohexose was shown by the isolation of 2-amino-2-deoxy-D-glucose (**4**) and a very small amount of paromamine (**5**). The paromamine was identified by a glc analysis of the O-pertrimethylsilyl-N-trifluoroacetyl derivative compared to an authentic sample. The isolated 2-amino-2-deoxy-D-glucose was characterized by comparison with authentic material by mixed melting point and optical rotation.

The aminopentose was also isolated after expenditure of considerable effort and the compound

shown to be 2-amino-2-deoxy-D-xylose (**6**). The 100 MHz PMR spectrum of a mutarotated anomeric mixture in D_2O gave the value of $J_{2'',3''}$ =9.5 Hz supporting either xylo- or arabino-stereochemistry. Final identification was made by optical rotation of the free base and optical rotation and melting point of the 2-acetamido derivative (**7**).

The 25 MHz CMR data obtained for seldomycin factor 1 free base (**1**) and paromamine free base (**5**) in D_2O solution are collected in Table 1. The CMR of paromamine has been previously assigned[5] and the agreement with published values is excellent. There is close correspondence in the chemical shifts of analogous carbons in paromamine and seldomycin factor 1 with the exception of C-5 and C-6 of 2-deoxystreptamine which show the expected upfield γ- and downfield β-effects due to 6-*O*-glycosidation.[5~7] The diagnostically useful β-protonation shifts[7,8] for those carbons situated adjacent to amino-bearing positions are also given in Table 1 and are in agreement with the assigned structure. These shifts permit unambiguous identification of the resonance of C-3''. An off-resonance single frequency decoupling experiment permits assignment of the C-5'' resonance which appears as a triplet. By the process of elimination the resonances of C-1'', C-2'' and C-4'' can be assigned.

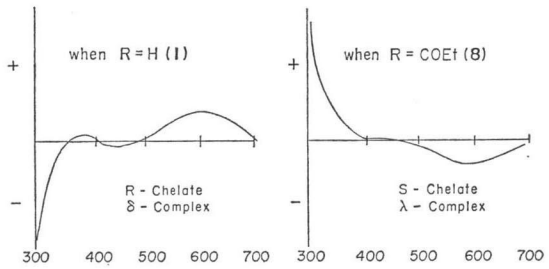
The chemical shift of C-4'' of the pentose in butirosin B[9] and ribostamycin A[5] are 81.8 and 83.4 ppm respectively, a result of substitution by the ring oxygen in these pentofuranoses. The analogous carbon in methyl α-D-xyloside, known to be present in the pentopyranose form, has a chemical shift of 69.4 ppm.[10] Therefore the 70.5 ppm shift of C-4'' in seldomycin factor 1 establishes that the aminopentose is present in the pyranose form.

Table 1. CMR Data of seldomycin factor 1 (**1**)

	Chemical shift*		
	Paromamine	Seldomycin factor 1	β-Shift
C-1′	101.9	101.6	3.4
C-2′	56.1	56.3	
C-3′	74.6	74.8	4.4
C-4′	70.8	70.8	
C-5′	73.8	73.8	
C-6′	61.6	61.7	
C-1	51.1	51.1	
C-2	36.7	36.7	7.6
C-3	50.3	50.2	
C-4	88.6	88.6	6.6
C-5	76.8	75.0	
C-6	78.4	87.0	6.8
C-1″		100.7	4.6
C-2″		56.3	
C-3″		74.7	4.3
C-4″		70.5	
C-5″		63.2	

* measured with reference to internal dioxane (67.4 ppm) and reported in ppm downfield from TMS.

Fig. 3. CD spectra of Cupra A complexes of seldomycin factor 1 free base (**1**) and its per-N-carboethoxy derivative (**8**).



Interestingly, the β-protonation shifts of the individual members of the pairs C-1′, C-1″ and C-4, C-6 are of nearly equal magnitude and do not show the contrast used in some cases to assign absolute stereochemistry to the sugars.[11] The failure of the empirical rule is likely a consequence of the presence of the novel 2''-amino group.

The absolute stereochemistry of seldomycin factor 1 follows from the CD spectra of the per-N-carbo-

Fig. 4. 100 MHz PMR spectrum of seldomycin factor 2 free base (**2**) in D_2O solution.

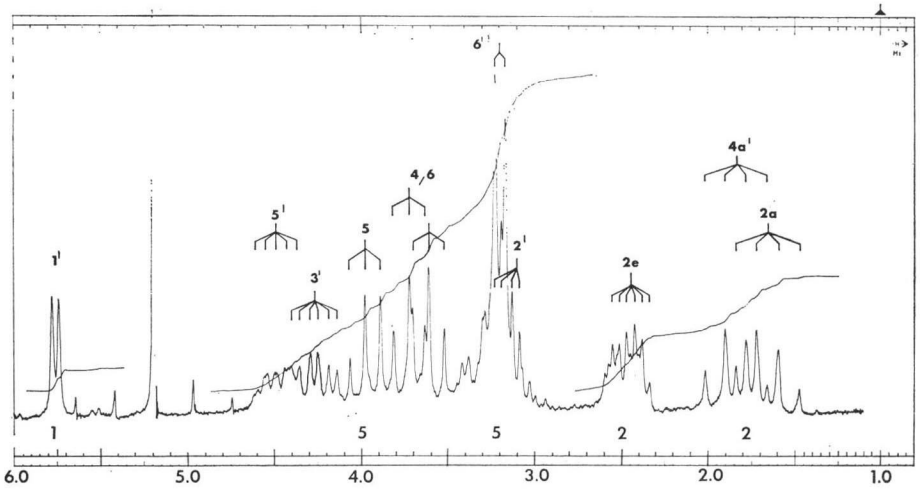


Table 2. PMR Parameters of seldomycin factor 2

Chemical Shifts (ppm)**			
H-1′	5.76	H-1	~3.3
H-2′	3.16	H-2a	1.65
H-3′	4.27	H-2e	2.45
H-4a′	1.84	H-3	~3.3
H-4e′	~2.5	H-4	3.61*
H-5′	4.49	H-5	3.98
CH_2-6′	3.20	H-6	3.72*
Coupling Constants (Hz)			
$J_{1',2'}$	4.0	$J_{1,2a}$	12.0
$J_{2',3'}$	10.2	$J_{1,2e}$	4.2
$J_{3',4a'}$	11.2	$J_{2a,2e}$	13.0
$J_{3',4e'}$	4.5	$J_{2a,3}$	12.0
$J_{4a',4e'}$	11.7	$J_{2e,3}$	4.2
$J_{4a',5'}$	11.7	$J_{3,4}$	9.0
$J_{4e',5'}$	2.0	$J_{4,5}$	9.0
J_{5',CH_2}	6.0	$J_{5,6}$	9.0
		$J_{1,6}$	9.0

* may be interchanged.

** reported in ppm downfield from *external* TMS—see experimental section.

Table 3. CMR Data of seldomycin factor 2*

	Seldomycin factor 2		Gentamine C_{1a}[6]	Neamine[7]	Nebramine[7]
	Chem. shift	β-Shift	**9**	**10**	**11**
C-1	51.2		51.3	51.4	51.3
C-2	36.6	7.6	36.7	36.5	36.8
C-3	50.2		50.5	50.3	50.1
C-4	88.3	11.1	88.3	87.7	87.8
C-5	76.9		76.9	76.9	76.9
C-6	78.4	5.0	78.4	78.1	78.6
C-1′	102.6	6.0	102.3	101.5	100.7
C-2′	57.6		50.7	56.2	50.4
C-3′	69.1	4.4	27.0	74.4	36.0
C-4′	36.9		28.5	72.4	67.1
C-5′	71.3	5.5	71.3	73.4	74.6
C-6′	45.7		46.0	42.6	42.7

* Chemical shifts are reported in ppm downfield from TMS but were measured with respect to internal dioxane (67.4 ppm). Because of a difference in the reference chemical shifts a 1.1 ppm correction has been added to the published[7] values of 10 and 11.

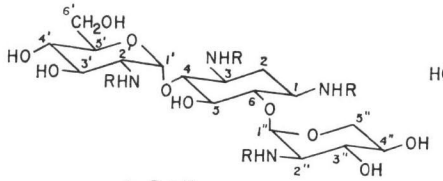
ethoxy derivative **8** in Cupra A solution[12,13] and from the isolation of 2-acetamido-2-deoxy-D-xylose from the degradation of seldomycin factor 1. In the Cupra A solution of seldomycin factor 1 per-N-carboethoxy derivative (**8**) complexation can only occur at the vicinal diols at C-3′, C-4′ and at C-3″, C-4″ and the measured spectra show an "S-chelate" which requires that glycols are both positioned with counterclockwise dihedral angles. A consideration of the projection formula of **8** requires that

both sugars and D-sugars. Interestingly, the CD spectra of the Cupra A solution of seldomycin factor 1 indicates "R-chelate" formation which must arise from clockwise dihedral angles. This implies that in the case of the parent antibiotic in Cupra A solution complexation occurs preferentially at the vicinal amino alcohols at C-2′, C-3′ and C-2″, C-3″.

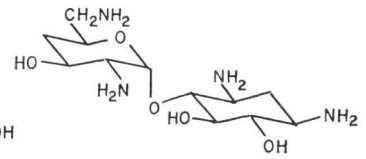
Taken together the PMR, mass spectrum, CMR, CD and isolated compounds define the structure of seldomycin factor 1 as 6-O-(2-amino-2-deoxy-α-D-xylopyranosyl) paromamine (**1**).

Structure of Seldomycin Factor 2

The 100 MHz PMR spectrum of a D_2O solution of seldomycin factor 2 free base (**2**) (Fig. 4) revealed only a single anomeric proton resonance indicating that this compound is a pseudo-disaccharide. Because of its reduced complexity the spectrum is completely interpretable (Table 2) and all chemical shifts and coupling constants can be determined. The high field chemical shifts of H-2′ and CH_2-6′,



1 R = H
8 R = CO_2Et



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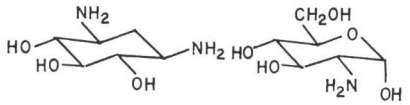
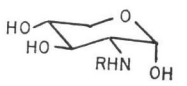
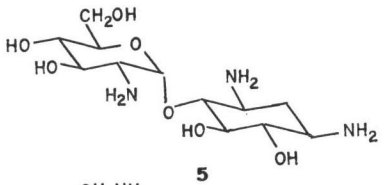


3 **4**



6 R = H
7 R = $COCH_3$



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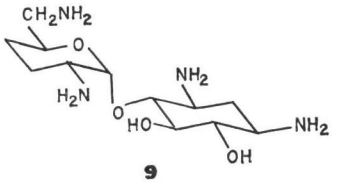

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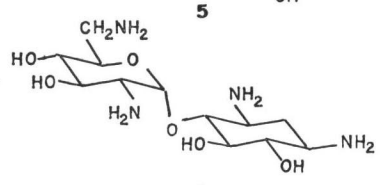

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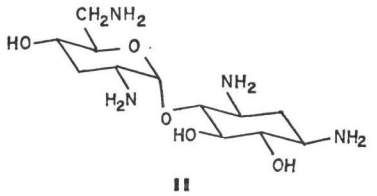
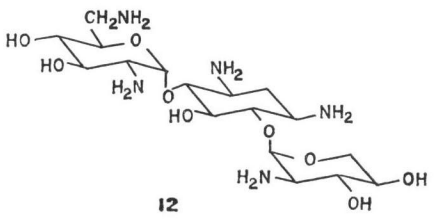


11



12

the additional multiplicity exhibited by the H-3′ and H-5′ resonances, and the presence of additional high field methylene resonances indicate that the sugar is a 2′,6′-diamino-2′,4′,6′-trideoxyhexose. The hexose ring proton coupling constants establish the configuration as xylopyranosyl and the anomeric proton coupling constant supports α-D-configuration. The chemical shifts and coupling constants of the protons associated with the cyclitol portion of the compounds are indicative of 2-deoxystreptamine.

The mass spectrum of seldomycin factor 2 free base (**2**) gave a protonated molecular ion at *m/e* 307 shown to have a $C_{12}H_{27}N_4O_5$ molecular formula under high resolution. The *m/e* 191, 173, 163, 145 tetrad of ions arising from 2-deoxystreptamine was found as was an ion at *m/e* 145 arising from the diaminohexose.

Carbon chemical shifts of seldomycin factor 2 free base were obtained from the 25 MHz CMR spectrum in D_2O solution and compared to published values for gentamine C_{1a} (**9**)[6], neamine (**10**)[7] and nebramine (**11**)[7] (Table 3). The agreement between the values for the 2-deoxystreptamine carbons is excellent and establishes the position of glycosidation of seldomycin factor 2. The diaminohexose chemical shifts were more difficult to compare; however, partial correlations were possible. The anomeric carbon chemical shift agreed well with each pseudo-disaccharide but was the closest to the value of gentamine C_{1a}. It is interesting to note that the C-4 chemical shift of **2** also agreed best with gentamine C_{1a} (**9**) supporting a closer conformational relationship about the glycoside bond with **9** than with either **10** or **11**. The C-2′ shift of **2** is best modelled in **10** which is also a compound with a 3′-hydroxyl group. There are no direct models for C-3′ of **2** in **9**～**11** although C-4′ of **11** has similar neighboring groups and a close chemical shift. Similarly C-4′ of **2** has no direct comparison save C-3′ of **11** and again the chemical shift agreement is good. The shifts of C-5′ and C-6′ are adequately modelled in **9**. The β-protonation shifts are also given in Table 3 and completely support the pattern of nitrogen substitution and chemical shift assignments.

Taken together these data support 4′-deoxyneamine (**2**) as the structure of seldomycin factor 2. After this determination was made, **2** was described by Bristol-Banyu Research Institute as a degradation product of 4′-deoxybutirosin.[14] Seldomycin factor 2 and this synthetic material, generously supplied by Dr. KAWAGUCHI, were compared by PMR, optical rotation, glc (*N*-TFA,*O*-TMS derivative), tlc (7 systems), mass spectrometry (*N*-Ac,*O*-TMS derivative) and antibiotic activity and found to be identical.

Probable Structure of Seldomycin Factor 3

Seldomycin factor 3 is an extremely rare component of the antibiotic mixture and has been available only for mass spectral determination. A protonated molecular ion at *m/e* 454 was observed and by high resolution peak matching found to have a $C_{17}H_{36}N_5O_9$ formula. Consideration of the fragment ions and comparison with seldomycin factor 1 indicated the presence of a diaminohexose. Particularly significant is the observation that the *m/e* 352 tetrad of ions from seldomycin factor 1 (Fig. 2) are all shifted by 1 amu to *m/e* 351 in seldomycin factor 3. On this basis the structure is proposed to be 6′-amino-6′-deoxy seldomycin factor 1 (**12**).

Experimental Part

Mass spectra were obtained on an A.E.I. MS-902 spectrometer at 50 eV using the direct insertion probe. PMR spectra were measured on a Varian Associates HA-100 spectrometer in D_2O solution.

Chemical shifts are reported in ppm downfield from external TMS contained in a co-axial capillary in the sample tube. Conversion to the commonly applied internal TSP scale in D_2O can be made by δ TMS (external)$=\delta$ TSP (internal)$+0.42$ ppm. CMR spectra were measured on a Varian Associates XL-100-15 spectrometer in D_2O solution. Chemical shifts were measured from internal dioxane (67.4 ppm) and are reported in ppm downfield from TMS. Melting points were determined with a Thomas-Hoover Uni-Melt apparatus. Optical rotations were measured with a Hilger and Watts polarimeter. CD spectra were obtained at 29°C (cell compartment temperature) in a Durrum-Jasco ORD/UV/CD 5 instrument operating under constant nitrogen flush. GLC analyses were performed on a Varian 1500 Dual Flame Ionization Gas Chromatograph using a DISC integrator for quantitation. The column was a 6 foot×2 mm I.D. glass packed with 3% Dexsil 300 GC on HP Chromosorb W 100~120 mesh maintained at 290°C and using helium as the carrier gas at 70 ml/minute.

Periodate Oxidation

A 10-mg sample of seldomycin factor 1 free base was dissolved in a mixture of 20 ml 0.15% periodic acid and 0.2 ml of concentrated sulfuric acid. The solution was allowed to react at 23°C for 72 hours. The reaction mixture was concentrated to a solid residue and subjected to acid hydrolysis with 6 N HCl at 110°C for 17 hours in a sealed tube. The oxidized, acid hydrolyzed material was compared with the acid hydrolyzed products of seldomycin factor 1 by TLC on cellulose in pyridine, ethyl acetate, acetic acid, water (35:35:7:21). Only 2-deoxystreptamine remained intact after oxidation and was visually equal in intensity to that present in the acid hydrolysate.

Acid Hydrolysis and Isolation of Paromamine

A 1.0-g sample of seldomycin factor 1 free base was suspended in 100 ml of 2 N HCl and refluxed for 12 hours. The solution was concentrated several times from water to remove excess HCl. The residue was dissolved in water and washed onto an AG 50 W-X2 (H^+) column which was developed with a gradient of water to 1 N HCl. Fractions of 8~10 ml were collected. Tubes 363~373 were concentrated to a residue, suspended in methanol and precipitated with acetone, collected and dried. An *O*-TMS-*N*-TFA derivative was prepared by the action of TriSil Z (Pierce Chemical Co.) and *S*-ethyl-trifluorothioacetate (Eastman) on a pyridine solution of the residue. The GLC spectrum of this material gave a single peak with a Rt of 451 sec which matched the Rt of a paromamine reference.

Acid Hydrolysis and Isolation of Amino Sugars

A water solution of 5.0 g of seldomycin factor 1 sulfate was converted to the free base and diluted with acid to give 6 N HCl and refluxed for 30 minutes. The hydrolysate was concentrated to a residue, redissolved in water and diluted with ethanol. A precipitate was removed by filtration and the filtrate was concentrated to a residue, redissolved in water and applied to a 1×30 cm column containing AG 50 W-X2 (H^+). Acid gradient was used for elution (water to 1 N HCl) and fractions of 5~8 ml were collected.

Tubes 101~105 contained 2-amino-2-deoxy-D-glucose hydrochloride. Equilibrated $[\alpha]_D^{22}+72.1°$, m.p. 185~187°C; literature[15] equilibrated $[\alpha]_D^{22}+72.5°$, m.p. 190~210°C. A mixed melting point showed no depression.

Tubes 107~115 contained 2-amino-2-deoxy-D-xylose hydrochloride. Equilibrated $[\alpha]_D^{22}+44.7°$; literature[15] equilibrated $[\alpha]_D^{22}+44.9°$.

Preparation of 2-Acetamido-2-deoxy-D-xylose

The aminopentose hydrochloride was dissolved in a mixture of 1 ml methanol and 10 ml water. Dowex 1×4 (CO_2^{-2}) 12 ml was added to the solution in an ice-bath. To the cooled solution was added 0.2 ml acetic anhydride and the solution was stirred for 2 hours. The resin was removed by filtration and the filtrate was percolated over IR 120 (H^+) to remove unreacted amine. The ninhydrin-negative effluent was concentrated and crystallized from ethanol, acetone, Skelly B. Equilibrated $[\alpha]_D^{22}+7.8°$, m.p. 189~191°C; literature[15] equilibrated $[\alpha]_D^{22}+9.0°$, m.p. 186~189°C.

Acknowledgements

The authors are indebted to Dr. STEPHEN HANESSIAN, University of Montreal, for a sample of paromamine and helpful discussions. The CMR data were available largely due to the efforts of Varian Associates and Drs. PAUL FAGERNESS and GEORGE GRAY as well as Dr. CHARLES MAJORS of the University of Utah. Numerous helpful discussions with Dr. PETER BEAK of the University of Illinois are gratefully acknowledged.

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