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A TRIMERIC L-RISTOSAMINE–AMMONIA CONDENSATION-PRODUCT

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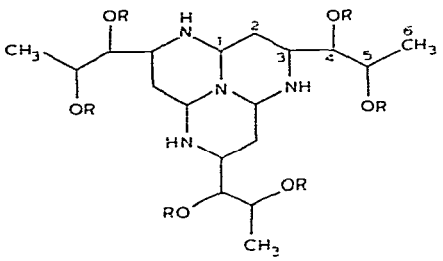
ABSTRACT

Three molecules of L-ristosamine (3-amino-2,3,6-trideoxy-L-*ribo*-hexopyranose) condense with one molecule of ammonia to give a stable compound [1,1′,1″-(dodecahydro-1,4,7,9*b*-tetrazaphenalene-2,5,8-trityl)tri-1,2-propanediol] reminiscent of the mode whereby formaldehyde and ammonia condense to form hexamethylenetetramine. The structure of the crystalline hexaacetate of this compound has been determined by X-ray crystallography. The mass-spectral fragmentation pattern and the ^{13}C-n.m.r. signals shown by the acetylated compound are assigned.

INTRODUCTION

Avoparcin, vancomycin, and ristocetin are important glycopeptide antibiotics. The structural elucidation of these complex natural products is an active field of research for a number of groups[1]. X-Ray crystallographic analysis has put the structure of vancomycin beyond doubt[2]. Both avoparcin and ristocetin contain 3-amino-2,3,6-trideoxy-L-ribo-hexopyranose (L-ristosamine), the structure of which was determined by Bognar *et al.*[1].

During hydrolytic studies on avoparcin, we followed the procedure of these authors to isolate ristosamine. Although a small amount of α,β-ristosamine was isolated, we found that most of the amino sugar existed under the conditions for processing of the hydrolyzate as a condensation product (**1**) of three moles of L-ristosamine and one mole of ammonia.



1 R = H
2 R = Ac

We were surprised that **1** was not observed by Bognar *et al.* in their isolation of ristosamine from ristocetin and we repeated their hydrolysis of ristocetin followed by the recovery process they outlined. In our hands, t.l.c. clearly showed **1** to be abundant in the ammoniacal eluate from the strong cation-exchange resin. The hydrolysis and an identical isolation-procedure were performed several times on avoparcin, and in each instance the major entity present was **1**, as opposed to free ristosamine.

DISCUSSION

Avoparcin contains one mole each of ristosaminylglucose, ristosamine, rhamnose, and mannose. Mild acid hydrolysis of the antibiotic complex may yield avoparcin CDP-1 and a mixture of the carbohydrate entities in the hydrolyzate. When this hydrolyzate is made neutral, the aglycon-related materials are largely precipitated. The resultant supernatant, if charged onto a strong cation-exchanger that is then eluted with dilute ammonia, would be expected to yield ristosamine and possibly some ristosaminylglucose. T.l.c. of this eluate showed a complex mixture containing one dominant component.

Further chromatography was used for partial resolution of the mixture. A portion of the ammoniacal eluate was lyophilized, charged onto a dextran-type strong cation-exchanger resin, and eluted with a gradient of aqueous ammonium formate. An early, major band from the resin (~50% of the load mass) was an unresolved mixture. The next band from the column was **1**, constituting ~25% of the load on the column. Well removed from this band, the third and minor product (10%) was eluted off; ^{13}C-n.m.r. spectroscopy showed this to be mostly a 7:3 mixture of the α and β anomers of ristosamine. A portion of the ammoniacal eluate of the cation-exchange resin was acetylated. The acetylated mixture was resolved by chromatography on dry silica gel to afford **2**. A solution of **2** in dichloromethane–hexane slowly yielded clumps of crystals.

TABLE I

CRYSTALLOGRAPHIC DATA FOR **2**

Formula	$C_{30}H_{48}N_4O_{12} \cdot CH_3CO_2H$
Mol. wt	716
Space group	Monoclinic, $P2_1$
a	11.892 (3) Å
b	12.719(8) Å
c	12.791(6) Å
β	104.73(3) deg.
Cell volume	1871 Å^3
Z	2
ρ obs (flotation in CCl_4/hexane)	1.257 g.cm^{-3}
ρ calc	1.271 g.cm^{-3}
Reflections measured	3091 ($3° < \theta < 60°$)
Unobserved $I < 2.0\sigma(I)$	755
Crystal size	700 × 210 × 150 μm

TABLE II

COORDINATES AND ANISOTROPIC THERMAL PARAMETERS OF THE NON-HYDROGEN ATOMS IN 2

Atom	*X*	*Y*	*Z*	*U11*	*U22*	*U33*	*U12*	*U13*	*U23*
C-1	0.6656	0.4375	0.2106	0.0232	0.0423	0.0250	0.0018	0.0081	0.0042
C-4	0.6364	0.4872	0.0982	0.0280	0.0380	0.0302	−0.0157	0.0011	−0.0003
C-7	0.7298	0.5123	0.2983	0.0332	0.0438	0.0392	−0.0073	0.0039	−0.0023
C-8	0.4069	0.2837	−0.0314	0.0266	0.0287	0.0299	−0.0028	−0.0014	−0.0015
C-9	0.5720	0.4090	0.0131	0.0223	0.0354	0.0354	−0.0048	0.0045	−0.0022
C-10	0.4866	0.3716	−0.1738	0.0222	0.0451	0.0324	−0.0099	0.0006	−0.0034
C-11	0.4967	0.3272	0.1602	0.0355	0.0290	0.0360	−0.0007	0.0056	−0.0006
C-13	0.7797	0.4651	0.4104	0.0495	0.0630	0.0289	−0.0269	0.0001	−0.0123
C-14	0.3825	0.2947	0.1868	0.0357	0.0466	0.0257	−0.0077	0.0066	0.0028
C-17	0.4636	0.4137	−0.2866	0.0361	0.0575	0.0263	−0.0157	0.0047	−0.0009
C-18	0 3803	0.3236	−0.1477	0.0339	0.0441	0.0289	−0.0021	0.0041	−0.0000
C-19	0.4429	0.3277	−0.3750	0.0367	0.0555	0.0405	−0.0152	0.0140	−0.0105
C-22	0.2530	0.1289	0.3228	0.0584	0.0693	0.0492	−0.0190	−0.0002	0.0190
C-26	0.5787	0.1856	−0.3352	0.0481	0.0776	0.0572	−0.0231	0.0098	−0.0114
C-28	0.3170	0.2139	0.1057	0.0324	0.0392	0.0313	−0.0023	0.0110	−0.0002
C-29	0.8734	0.6449	0.2938	0.0398	0.0485	0.0493	−0.0070	−0.0106	0.0063
C-31	0.2021	0.0968	0.2055	0.0326	0.0443	0.0545	−0.0025	0.0146	0.0169
C-32	0.2001	0.1821	0.1205	0.0288	0.0435	0.0339	−0.0017	0.0045	0.0023
C-35	0.0603	−0.0275	0.2270	0.0427	0.0490	0.0453	−0.0125	0.0095	−0.0047
C-36	0.6930	0.4241	0.4687	0.0525	0.0833	0.0328	−0.0260	0.0033	−0.0002
C-40	−0.0680	−0.0431	0.2194	0.0417	0.0781	0.0878	−0.0137	0.0260	0.0071
C-41	0.6993	0.1505	−0.3315	0.0553	0.0856	0.1213	0.0114	0.0260	−0.0173
C-43	0.3974	0.3688	−0.4874	0.0648	0.1070	0.0365	−0.0011	0.0084	0.0087
C-47	0.0629	0.3243	0.0735	0.0499	0.0641	0.0585	0.0007	0.0169	0.0281
C-53	0.2007	0.6691	−0.0070	0.0554	0.0514	0.1187	0.0195	0.0389	0.0022
C-57	1.0372	0.3010	0.4160	0.0756	0.1189	0.1131	0.0362	−0.0062	0.0126
C-58	0.9715	0.3946	0.4362	0.0419	0.1033	0.0551	0.0056	0.0043	0.0210
C-59	0.0122	0.4187	0.1151	0.0771	0.0727	0.1299	0.0270	0.0321	−0.0092
C-63	0.3654	0.5784	−0.3397	0.0573	0.0489	0.0569	−0.0058	0.0077	0.0052
C-67	0.2535	0.6332	−0.3530	0.0653	0.0670	0.0741	0.0112	0.0047	0.0228
C-79	0.2987	0.5937	−0.0088	0.0382	0.0456	0.0562	−0.0018	−0.0031	−0.0023
C-80	0.9807	0.6652	0.2608	0.0577	0.0695	0.0978	−0.0275	0.0117	0.0044
N-1	0.4656	0.3698	0.0450	0.0238	0.0295	0.0310	−0.0046	0.0042	−0.0069
N-2	0.3006	0.2487	−0.0056	0.0273	0.0489	0.0284	−0.0108	0.0010	0.0025
N-3	0.5412	0.4515	−0.0935	0.0292	0.0474	0.0253	−0.0091	0.0024	0.0017
N-4	0.5585	0.4022	0.2360	0.0320	0.0466	0.0272	−0.0107	0.0050	−0.0097
O-1	0.8298	0.5489	0.2606	0.0327	0.0398	0.0372	−0.0058	0.0022	−0.0045
O-2	0.8569	0.3795	0.4013	0.0368	0.0568	0.0427	−0.0039	−0.0042	0.0010
O-3	0.0402	0.3005	−0.0202	0.0710	0.1178	0.0484	0.0400	0.0075	0.0279
O-4	0.5554	0.2829	−0.3661	0.0480	0.0527	0.0591	−0.0068	0.0298	−0.0061
O-5	0.8284	0.7018	0.3458	0.0474	0.0464	0.0693	−0.0101	0.0013	−0.0148
O-6	0.3610	0.4783	−0.3051	0.0323	0.0507	0.0418	−0.0008	0.0091	0.0069
O-7	0.0807	0.0694	0.1926	0.0274	0.0473	0.0535	−0.0052	0.0074	0.0174
O-8	0.1351	−0.0899	0.2611	0.0472	0.0563	0.0870	−0.0119	0.0050	0.0283
O-9	0.5100	0.1290	−0.3090	0.0673	0.0768	0.1657	−0.0067	0.0193	0.0397
O-11	0.1389	0.2725	0.1504	0.0443	0.0392	0.0419	0.0073	0.0097	0.0056
O-12	0.3001	0.5092	0.0405	0.0535	0.0571	0.0788	0.0082	0.0317	0.0123
O-13	0.3706	0.6202	−0.0601	0.0497	0.0492	0.0884	0.0000	0.0139	0.0060
O-14	1.0149	0.4769	0.4753	0.0470	0.1115	0.1268	−0.0209	−0.0097	−0.0020
O-15	0.4496	0.6154	−0.3586	0.0713	0.0613	0.1548	−0.0181	0.0218	0.0367

TABLE III

COORDINATES AND ISOTROPIC THERMAL PARAMETERS OF THE HYDROGEN ATOMS IN **2**

Atom	*X*	*Y*	*Z*	*U*
H-1	0.3866	0.2690	−0.3694	0.0418
H-2	0.5310	0.4540	−0.2987	0.0034
H-3	0.8048	0.5055	0.4533	0.0882
H-4	0.6709	0.5772	0.3084	0.0723
H-5	0.2547	0.0382	0.2035	0.0576
H-6	0.1541	0.1477	0.0429	0.0596
H-7	0.7189	0.3857	0.2158	0.0698
H-8	0.6246	0.3479	0.0186	0.0105
H-9	0.5392	0.3247	−0.1619	0.0271
H-10	0.4645	0.2139	−0.0215	0.0365
H-11	0.3554	0.1605	0.1112	0.0453
H-12	0.5450	0.2678	0.1621	0.0369
H-13	0.5740	0.5680	0.0872	0.0509
H-14	0.7107	0.5015	0.0712	0.0329
H-15	0.3985	0.2638	0.2612	0.0796
H-16	0.3353	0.3651	0.1786	0.4667
H-17	0.3540	0.2657	−0.1933	0.0396
H-18	0.3197	0.3649	−0.1629	0.0201
H-19	0.2476	0.2643	−0.0270	0.0616
H-20	0.5013	0.4870	−0.1109	0.1195
H-21	0.5308	0.4306	0.2638	0.0859
H-23	0.2020	0.6023	−0.3980	0.0633
H-24	0.6598	0.3540	0.4363	0.0633
H-25	0.7455	0.3916	0.5424	0.0633
H-26	0.2374	0.6285	−0.2987	0.0633
H-27	0.3239	0.1327	0.3483	0.0633
H-28	1.0199	0.7164	0.3116	0.0633
H-29	−0.1278	−0.0462	0.2856	0.0633
H-30	0.6386	0.4465	0.4556	0.0633
H-32	0.2425	0.7105	−0.4043	0.0633
H-33	1.0258	0.6316	0.2639	0.0633
H-34	0.7616	0.2105	−0.2971	0.0633
H-35	0.4498	0.3947	−0.5343	0.0633
H-36	1.0077	0.2368	0.3961	0.0633
H-37	0.2245	0.2105	0.3499	0.0633
H-38	0.0847	0.5000	0.1609	0.0633
H-39	0.2566	0.0526	0.3695	0.0633
H-40	0.9767	0.6842	0.1916	0.0633
H-41	0.3233	0.3978	−0.4936	0.0633
H-42	1.0633	0.3158	0.4634	0.0633

X-Ray crystallography. — A suitable crystal was cut from a clump so that recrystallization was not necessary. Unit-cell dimensions were determined from least-squares refinement of data for 25 reflections ($20° < \theta < 30°$) aligned manually on the diffractometer. Intensities were measured by using the $\theta/2\theta$ scan-method with an Enraf–Nonius CAD-3 diffractometer (nickel-filtered CuKα radiation). No absorption corrections were applied. Crystal data are presented in Table I. Tables II

and III include co-ordinates and temperature parameters for non-hydrogen and hydrogen atoms.

At the outset of the analysis, the calculated molecular weight for the asymmetric unit (mol. wt. = 708 for $Z = 2$) could not be reconciled with the expected chemical formula ($C_{12}H_{19}NO_6$) of acetylated ristosamine. Consequently the first MULTAN[3] calculation was made by assuming that the asymmetric unit would contain ~50 carbon atoms. A partial structure consisting of 33 peaks, 19 in a polycyclic fragment, was eventually found as the fourth most-probable solution in a calculation involving 450 reflections ($E \geq 1.38$) using five phases to generate 256 solution-sets. The 19-atom fragment was used as a known group in a further MULTAN calculation, which yielded an extended, partial structure of 47 atoms (17 closed) in the most-probable solution. From this starting point, the complete trial structure was obtained from structure-factor and difference electron-density calculations. At each stage, the partial structure included was refined isotropically before a difference map was computed to reveal new atom sites. It was concluded that the structure had been completely established when no new peaks appeared in the difference map. Analysis of the refined, isotropic temperature-parameters (all atoms were assumed to be carbon) and bond distances, keeping in mind that the compound might be related to ristosamine, allowed the structure to be identified as the acetic acid salt of a condensation product between three diacetylated ristosamine molecules and a molecule of ammonia, corresponding to the formula $C_{30}H_{48}N_4O_{12} \cdot CH_3CO_2H$.

Anisotropic refinement after assignment of atom types led to $R = 0.083$; when hydrogen atoms were included, the final discrepancy factor was $R = 0.07$. All calculations were made by using the XRAY76 set of crystallographic programs[4]. It may

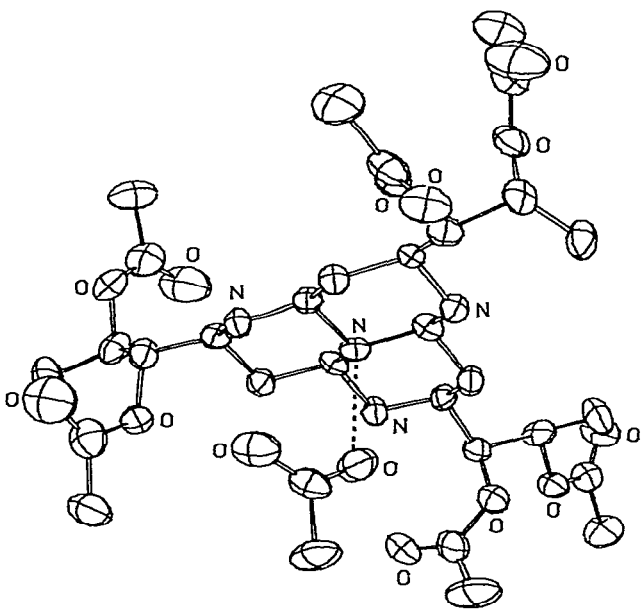


Fig. 1. ORTEP diagram of 2.

be seen by inspection of a model that all three ristosamine residues have the same relative configuration. However, an attempt to establish the absolute configuration of the molecule by the anomalous-dispersion effect of oxygen was inconclusive. An ORTEP diagram of the structure is shown in Fig. 1; the interaction between the

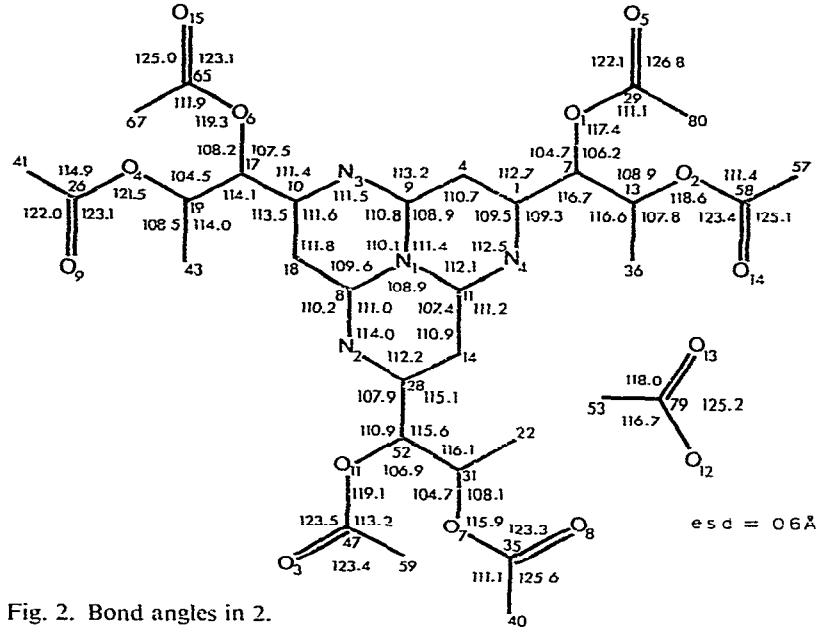


Fig. 2. Bond angles in 2.

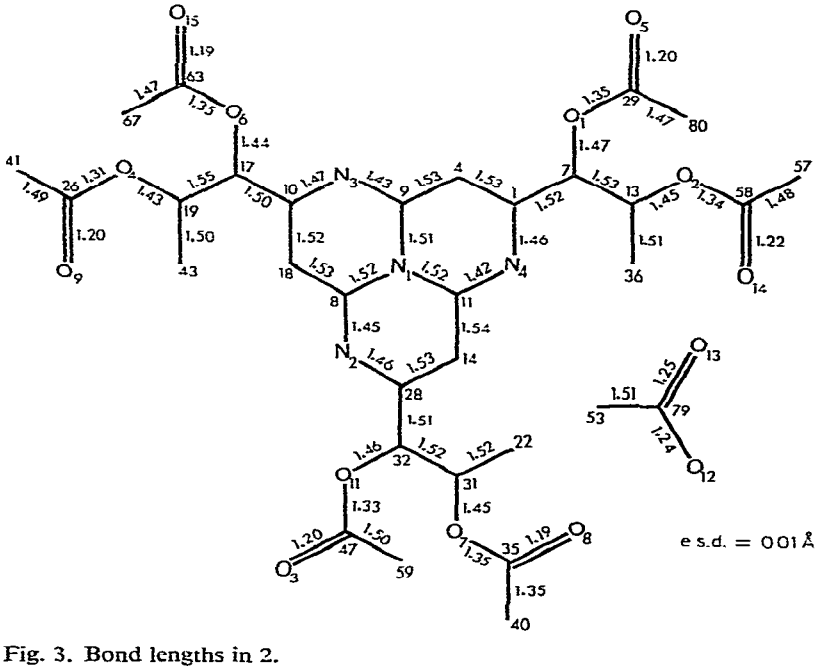
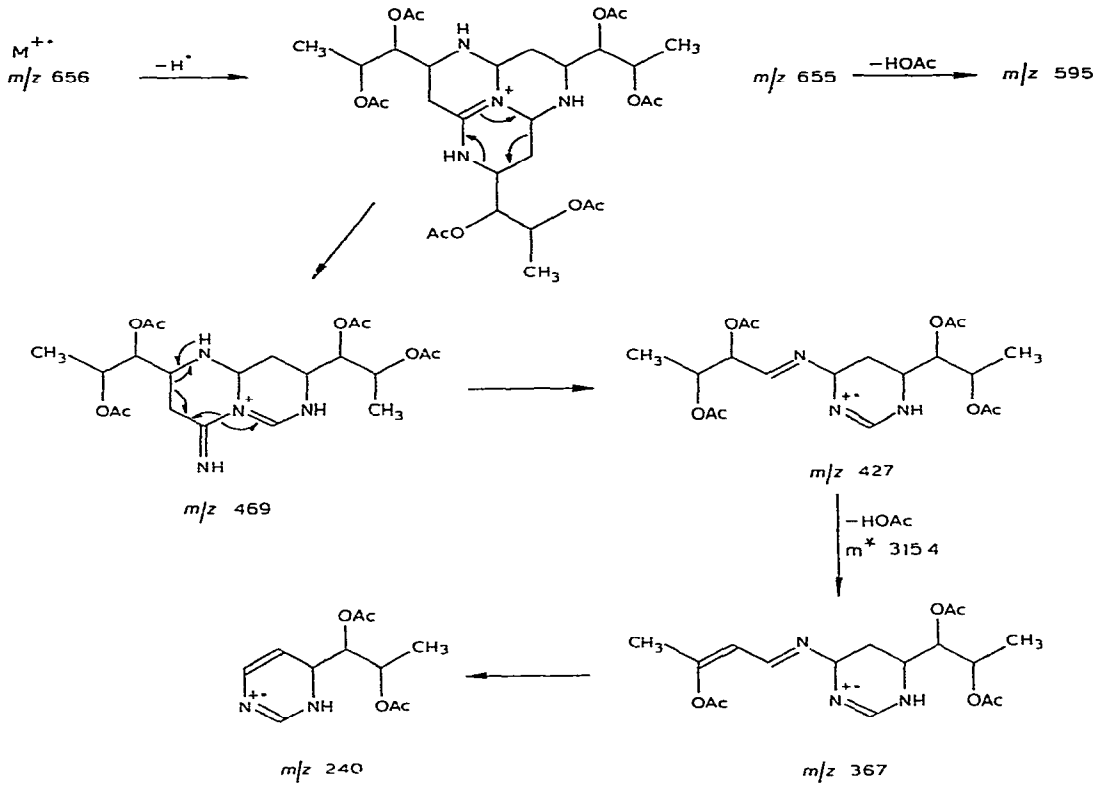


Fig. 3. Bond lengths in 2.



Scheme I. Mass-spectral fragmentation pattern of 2

TABLE IV

HIGH-RESOLUTION MASS-SPECTRAL VALUES FOR 2

Measured mass	*Calculated mass*	*Composition*
655.3164	655.3190	$C_{30}H_{47}N_1O_{12}$
595.2966	595.2979	$C_{29}H_{43}N_1O_{10}$
469.2312	469.2298	$C_{21}H_{33}N_1O_8$
367.1789	367.1743	$C_{17}H_{25}N_3O_6$

acetic acid molecule and the central nitrogen atom is indicated by the dotted line. Figs. 2 and 3 show bond angles and bond lengths, respectively, in **2**, as determined by X-ray analysis.

Mass-spectral studies on **2**. — The mass spectrum of **2** showed an intense peak at *m/z* 655 corresponding to M − H·. Loss of a neutral $C_9H_{14}O_4$ fragment by a retro Diels–Alder process afforded an ion at *m/z* 469, which then lost C_2H_4N to give *m/z* 427. Further fragmentation gave significant peaks at *m/z* 367 and 240. A resonable interpretation of the fragmentation pattern is shown in Scheme I.

TABLE V

N.M.R. ASSIGNMENTS FOR **1** AND **2**

Atom no.	**1**			**2**		
	^1H	^{13}C	J	^1H	^{13}C	J
6	1.18	19.99	118.5	1.2	13.7	15.4
2	2.5	28.74	121.5	2.0	35.1	
3	2.5	55.13	131	2.8	53.5	22.8
1	4.1	67.67	137	3.0	72.0	24.9
4	4.65	75.51	138	4.9	75.0	39.0
5	5.0	71.60	141.5	5.2	68.6	40.3

Four significant signals were peak-matched, so that observed and calculated values could be compared as shown in Table IV.

N.m.r. studies on **1** *and* **2**. — The ^{13}C-n.m.r. spectrum of **1** displayed the expected six signals. Initially, we were surprised at the absence of an anomeric carbon signal, which is usually observed between 90 and 100 p.p.m. The corresponding six signals were observed in **2**, together with exceptionally intense signals at 20.6, 20.8, 169.5 and 169.9 p.p.m. corresponding to the acetate methyl and carbonyl groups, respectively. A weak signal at 172 p.p.m. corresponds to the carbonyl carbon of the solvate molecule of acetic acid. Because of the X-ray solution of the structure, only the assignment of the ^{13}C signals remained.

The assignment of proton chemical-shifts for **1** and **2** were straightforwardly based upon interpretation of coupling patterns and observation of shifts caused by acetylation. These values, together with off-resonance, residual coupling-constants, were used to assign the ^{13}C spectral peaks as shown in Table V.

As C-6 and C-2 gave rise to a quartet and a triplet, respectively, in the off-resonance spectrum, these assignments were obvious. With the decoupler offset towards higher field, residual splitting should increase with decreased shielding of the protons attached to the relevant carbon. This implies that J_{CH} for all six carbons is approximately the same with the decoupler set in the normal position[5]. Consequently, the smallest coupling-constant should be associated with the methyl carbon atom, whereas the largest should be connected with C-5. In this manner, the remaining ^{13}C assignments of Table V were made.

The condensation artifact. — Formaldehyde and ammonia react to yield the well-known hexamethylenetetramine. The X-ray crystallography work of Dickinson and Raymond on this compound gave the first complete solution of an organic structure by this technique[6]. Formation of a condensation product of aminodeoxy sugars and ammonia does not previously appear to have been reported. We have not tested the generality of our observation with other 3-amino-2,3-dideoxy sugars.

The neutralized hydrolyzate from avoparcin was processed without the use of a strong cation-exchange resin, with subsequent elution with ammonia. The material

was charged onto a dextran weak-based, cation-exchange resin and eluted by using a gradient between two dilute solutions of sodium chloride. Under these conditions, no trace of **1** was detected. Neutral sugars were washed through the column as a discrete band. Later fractions contained ristosamine hydrochloride, again as a 7:3 mixture of α and β anomers. This observation indicates that the condensation between L-ristosamine and ammonia occurs on the strong cation-exchange resin. The ideal conditions for complete condensation between these two molecules have not been investigated. The relationship between **1** and **2** was established conclusively by deacetylation of **2** with ammonia in methanol to give crystalline **1**.

EXPERIMENTAL

General methods. — Mass-spectral studies were performed with an AEI MS-9 mass spectrometer. The ^{13}C-n.m.r. spectrum of **1** was taken in D_2O (25.5 MHz) with a Varian XL-100 instrument. The offset for decoupling studies was 7000 Hz. The spectrum of **2** was taken in Me_2SO-d_6 on the same instrument; the offset for decoupling was 600 Hz.

T.l.c. was conducted on silica gel with 1:1:1:1 *tert*-butyl alcohol–ethyl acetate–acetic acid–water, and sulfuric acid charring was used for detection. Neutral sugars appeared as black spots almost at once. Rhamnose was the exception, in that it gave a yellowish tinge. Ristosamine appeared as a brownish spot a little later. To detect **2**, charring had to be continued for > 10–12 min to give a brownish grey spot at $R_F \sim 0.3$. Ristosamine and the neutral sugars exhibited R_F values in the range 0.6–0.8.

Mild acid hydrolysis of avoparcin. — Avoparcin (~100 g) was dissolved in 500 mL of 0.5M hydrochloric acid and heated for 1.75 h on a steam bath. The solution was cooled and the pH adjusted to 7.4 with Dowex-X2 (OH^-). The resin and other insoluble material were filtered off and the yellow filtrate was allowed to percolate through a column of Dowex 50W-2X (NH_4^+) resin. The column was washed with water and eluted with 3% ammonium hydroxide. The eluate was concentrated and freeze-dried to give 4.9 g of solid, ~1.4 g of which was charged onto ~100 mL of SP Sephadex (NH_4^+). The column was developed by using a gradient between 0.05 and 0.5M ammonium formate. Fractions (25 mL) were collected and pooled on the basis of t.l.c. work (silica gel). Combinations were made and the solutions concentrated and freeze-dried to yield the following amounts: A, fractions 3–7, 765 mg; B, fractions 9–12, 78 mg; C, fractions 13–15, 319 mg; and D, fractions 53–56, 137 mg.

Preparation C yielded exceptionally clear ^{1}H- and ^{13}C spectra, the assignments of which are cited in Table V under **1**.

The hydrochloride of this compound was prepared by dissolving it in methanol and adding a small amount of acetyl chloride. After a short period, an excess of ether was added to yield a suspension. The solid was recovered and dried to yield a white solid; $[\alpha]_D^{25}$ +23 ±2° (*c* 1.048, water).

Anal. Calc. for $C_{18}H_{36}N_4O_6 \cdot 3\ HCl \cdot 2\ H_2O$: C, 39.31; H, 7.83; N, 10.19; Cl, 19.38. Found: C, 39.63; H, 7.58; N, 10.00; Cl, 19.50.

Preparation D was examined by ^{13}C-n.m.r. spectroscopy. The spectrum showed six strong signals at 17.5, 31.8, 49.4, 66.4, 68.4, and 90.5 p.p.m., which are assigned to C-6, C-2, C-3, (C-4 and C-5), and the anomeric C-1 of the α anomer of ristosamine, respectively. The spectrum also exhibited the following signals of ~25% the intensity of the stronger signals: 18.6 and 19.8; 31.0 and 38.5; 51.6 and 54.5; 72.2 and 76.8; and 86.5 and 98.7 p.p.m.

In another experiment, avoparcin (50 g) in (250 mL) of M hydrochloric acid was heated for 50 min on a steam bath. The pH was then adjusted to pH 6.5 with Dowex 1-X4 (OH^-) resin. The resin and precipitated material were filtered to give a yellow filtrate that was allowed to percolate through a 1.9-cm diameter column of Dowex 50W-X4 (H^+) (bed depth 51 cm). The column was washed with water and eluted with 2% ammonium hydroxide solution. The eluate was concentrated and freeze-dried to give 3.2 g of solid, ~2.5 g of which was acetylated overnight at room temperature in 30 mL of acetic anhydride and 1 mL of pyridine. The excess of reagent was decomposed by addition of methanol (60 mL). The solvent was evaporated to a gum that was charged onto a dry column of silica gel. The column was developed with ethyl acetate and 150-mL fractions were collected. The third fraction was concentrated to yield 300 mg of **2**. Again, clear ^1H- and ^{13}C-n.m.r. spectra were obtained on this material, and the mass-spectral studies were also conducted with this preparation.

Crystals of **2** *for X-ray studies.* — Avoparcin (~200 g) in 1 L of 0.5M hydrochloric acid was heated for 2.5 h on a steam bath. The cooled solution was adjusted to pH 7.4 with Dowex 1-X2 (OH^-). The resin plus insoluble material was filtered off. The filtrate was percolated through a column (3,8 diameter and 61 cm bed depth) of Dowex 50W-X2 (NH_4^+). The column was washed with water and then eluted with 5% ammonium hydroxide solution. Fractions of 250 mL were collected. Fraction 3 yielded 18.5 g upon concentration and freeze drying. This material (~10 g) was acetylated overnight in 125 mL of acetic anhydride with 5 mL of pyridine added. After removal of the solvent, the residue was charged onto a dry column of silica gel (5 cm diameter and 112 cm bed depth). The column was developed with 500 mL of chloroform and then with 80:1 ethyl acetate–methanol. The volume of each fraction collected was 150 mL. Concentration of fraction 10 yielded a solid that crystallized from dichloromethane–hexane. A first crop of 150 mg of white crystals of **2** was obtained, m.p. 110°, $[\alpha]_D^{25}$ −20 ±1° (*c* 0.836, methanol); R_F on silica gel, 0.75 with 80:1 ethyl acetate–methanol.

Anal. Calc. for $C_{30}H_{48}N_4O_{12} \cdot CH_3CO_2H$ (716): C, 53.63; H, 7.26; N, 7.82. Found: C, 53.56; H, 7.35; N, 7.68.

Conversion[7] *of* **2** *into* **1**. — Crystalline **2** (~350 mg) was dissolved in 50 mL of methanol and ammonia gas was bubbled in for one h. The solution was kept for 2 days and then the solvent was evaporated to low volume. Scratching the inside of the flask produced crystals that were recrystallized from methanol–ether to afford 160 mg of white crystals, m.p. 245–247°. Spectral and t.l.c. data for these crystals were identical to those already described for **1**; $[\alpha]_D^{25}$ +28 ±2 (*c*, 0.78, water).

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