Structure and Stereochemistry of Ristosamine

R. Bognar and F. Sztaricskai

Institute of Organic Chemistry, L. Kossuth University, 4010 Debrecen, Hungary

M. E. Munk*

Arizona State University, Tempe, Arizona 85281

J. Tamas

Central Chemical Research Institute, Hungarian Academy of Sciences, 1088 Budapest, Hungary

Ristosamine, an amino sugar derived from the antibiotic ristomycin A, is shown to be the previously unreported 3-amino-2,3,6-trideoxy-L-*ribo*-hexopyranose.

Ristomycin, an antibiotic produced by *Proactinomyces fructiferi var. ristomycin,*[1] exhibits a wide antibacterial spectrum[2] and can be classified among the vancomycin-type antibiotics.[3] Ristomycin is composed of the variants A and B, each of which gives rise to the same, previously unreported, ninhydrin-positive, reducing deoxy amino sugar under conditions of acidic or alkaline hydrolysis.[4]

In a previous report[5] we have described the neutral carbohydrates attached to ristomycin A aglycone, and the partial structure of the latter. The present study focuses on the investigation of the structure of the trideoxyaminohexose ristosamine (1), isolated after hydrolysis of ristomycin A with 0.5 *N* hydrochloric acid at 100° (Scheme I).

Ristosamine hydrochloride, $C_6H_{13}O_3N \cdot HCl$, gives positive Fehlings, Tollens, Keller–Kiliani, and iodoform reactions, as well as the xanthydrol color test. Oxidation with periodic acid[6] leads to the consumption of approximately 2 mol of the reagent. The formation of formaldehyde could not be detected; however, acetaldehyde is distilled from the reaction mixture and identified as its 2,4-dinitrophenylhydrazone. This observation and the positive iodoform test point to a 6-deoxy sugar.

Although direct conversion of ristosamine to its methyl glycoside could not be achieved, the acidic methanolysis of ristomycin A produced methyl ristosaminide, most conveniently isolated as its crystalline hydrochloride salt (2). The consumption of 0.7 mol of sodium periodate by this compound indicates a pyranoside ring and is consistent with a 2,6 or a 4,6 dideoxy sugar. The formation of acetaldehyde upon periodate cleavage of 1 requires the former, *i.e.*, C-4 must bear either the hydroxyl or amino group. A

2-deoxy sugar is in accord with the positive Keller–Kiliani and xanthydrol tests and the double-resonance nmr studies, which demonstrate spin coupling between the anomeric and methylene hydrogens.
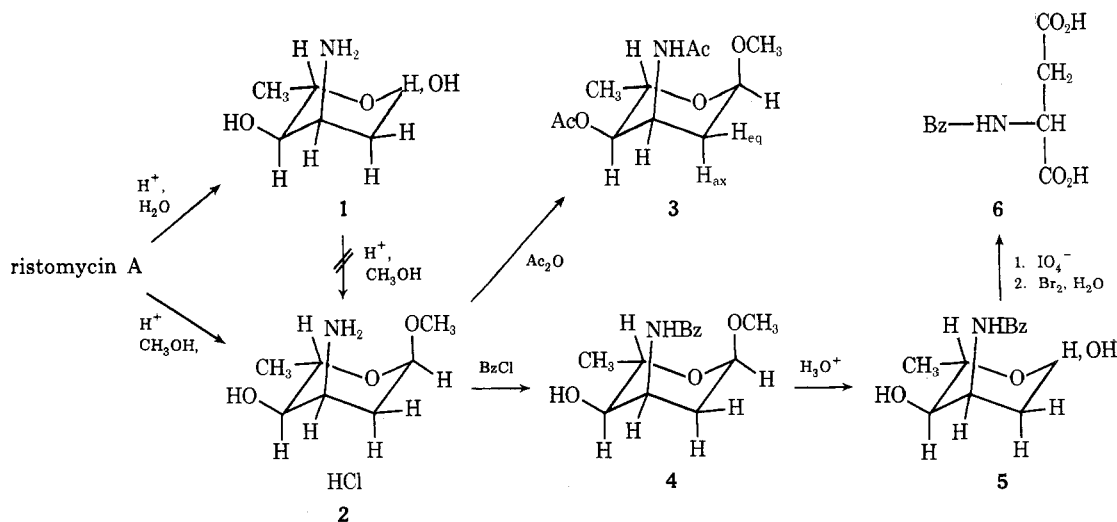
Methyl ristosaminide hydrochloride could be directly converted to methyl *N,O*-diacetylristosaminide (3) and methyl *N*-benzoylristosaminide (4). Acid hydrolysis of the latter yields crystalline *N*-benzoylristosamine (5). Treatment of compound 5 with sodium periodate followed by further oxidation with aqueous bromine leads to the isolation of crystalline *N*-benzoyl-D(−)-aspartic acid (6). The amino group of ristosamine must therefore reside at C-3. These chemical data permit the assignment of the 2,3,6-trideoxy-3-aminohexopyranose structure 1 to ristosamine.

This assignment finds support in the electron-impact studies of compounds 2 and 3 at 70 eV (Table I). The mass spectrum of the hydrochloride salt 2 displays a negligible relative abundance (0.1%) of the molecular ion of the free base at *m/e* 161 and the expected weak peaks at *m/e* 143, 130, 129, and 111 corresponding to M − H_2O, M − OCH_3, M − CH_3OH, and M − (H_2O + CH_3OH), respectively.

Barring deep-seated skeletal rearrangement, which is not considered likely,[7] the remaining prominent peaks in the mass spectrum of compound 2 provide structural information at two levels: that required by the data and that consistent with the data. In the former category, ion *m/e* 117, $C_5H_{11}NO_2$ by high resolution, is M − CH_3CHO, a finding consistent only with a 6-deoxy sugar. Likewise, ion *m/e* 59a, C_2H_5NO, requires hydroxyl and amino groups on adjacent carbon atoms.

The appearance of these two ions narrows the plausible

Scheme I

	Compound 2			Compound 3	
m/e	$\%^a$	Ion composition[b]	m/e	$\%^a$	Ion composition[b]
161 (M·⁺)	0.1		214	2	$C_{10}H_{16}NO_4$
143	11	$C_7H_{13}NO_2$	213	1	
130	20	$C_6H_{12}NO_2$	185	4	$C_9H_{15}NO_3$
129	3		153	22	$C_8H_{11}NO_2$
117	7	$C_5H_{11}NO_2$	143	10	$C_6H_9NO_3$
111	3		142	19	$C_7H_{12}NO_2$
104	27	$C_4H_{10}NO_2$	128	14	$C_6H_{10}NO_2$
100	11	$C_5H_8O_2$	117	6	$C_5H_{11}NO_2$
86	55	C_4H_8NO	115	6	$C_5H_9NO_2$
72	77	C_3H_6NO	101	25	$C_4H_7NO_2$
59	100 { a 60	C_2H_5NO	86	25	
	b 40	C_3H_7O	72	14	
58	85	C_3H_6O	59	15	
44	84		58	14	
43	53		43	100	
38	25	HCl			
36	74	HCl			

a Peak intensity relative to base peak. *b* Derived from high-resolution studies.

molecular structures of methyl ristosaminide to four: 2,3,6-trideoxy-3-amino-, 2,4,6-trideoxy-4-amino-, 3,4,6-tri-deoxy-3-amino-, and 2,4,6-trideoxy-2-aminohexopyranos-ides. Ion m/e 86 (C_4H_8NO) is inexplicable in terms of the last-named structure, but each of the other three is compatible with the major ions in the mass spectra of compounds **2** and **3**. Only the first-named compound, however, fits the nmr and chemical evidence as well. Scheme II suggests the origin of the structurally significant ions expected from this compound.

Scheme II
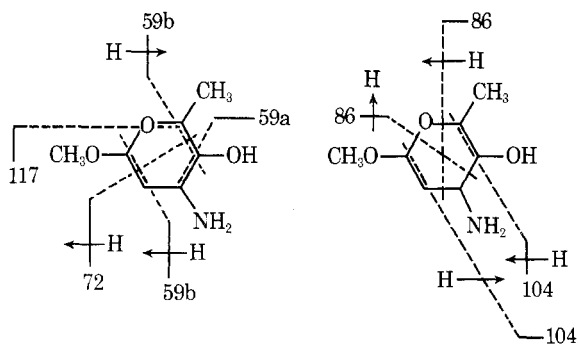
The isolation of *N*-benzoyl-D(−)-aspartic acid (**6**) from the oxidative cleavage of compound **5** suggests the *R* configuration at C-3 of ristosamine. Comparing the molecular optical rotation value of **1** (MD −62.97°) to those of the α and β equilibrium mixtures of the eight possible 2,6-dideoxyhexoses, only the L-ribo compound, L-digitoxose, shows a comparable sign and magnitude of rotation, MD −68°.[8,9]

The nmr spectrum of crystalline methyl *N*,*O*-diacetylris-tosaminide (**3**) at 220 MHz provides strong confirmation of the structure and configuration proposed.

The low-field doublet at δ 4.79 is assigned to the anomeric hydrogen H-1. The low values for both $J_{1,2ax}$ (4 Hz) and $J_{1,2eq}$ (<1 Hz) require an equatorial position for H-1, *i.e.*, crystalline **3** is the α anomer. Irradiation of signal H-1 results in the collapse of two higher field signals: the broadened doublet of doublets at δ 1.89 (H-2eq) to a sharp doublet of doublets (*J* = 14, 2.5 Hz), and the doublet of triplets at δ 2.09 (H-2ax) to a doublet of doublets (*J* = 14, 3.5 Hz). The observed coupling of H-1 to two other hydrogens es-

tablishes ristosamine as a 2-deoxy sugar. In addition, the low $J_{2ax,3}$ value (3.5 Hz) excludes a H-2ax–H-3ax arrangement; therefore, the acetamido group at C-3 must occupy an axial position.

The assignment of the higher field signal (δ 1.89) of the geminal hydrogens at C-2 to the equatorially rather than the axially disposed hydrogen is in contrast to the general view.[10] While not secure, this assignment is believed likely, and has as its basis the observation that the higher field signal at δ 1.89 displays a smaller coupling (*J* = <1 Hz) with the anomeric hydrogen H-1 than the lower field methylene hydrogen signal at δ 2.09 (*J* = 4.0 Hz).

In those α-2-deoxyhexopyranoses that have been thoroughly studied by nmr, $J_{1eq,2eq}$ is always less than or equal to $J_{1eq,2ax}$. In the nmr spectrum of methyl α-*N*,*O*-dibenzoylvancosaminide, the H-2ax signal is assigned a higher field position than H-2eq, and $J_{1eq,2ax}$ = 4.5 Hz while $J_{1eq,2eq}$ = 0 Hz.[11] Similarly, in the spectrum of methyl α-D-chromoside C, the H-2ax signal is reported upfield of H-2eq, and $J_{1eq,2ax}$ = 3.5 and $J_{1eq,2eq}$ = 1.5 Hz.[12] The axial hydrogen at C-2 of methyl 2-deoxy-D-*arabino*-hexopyranose appears at higher field and $J_{1eq,2ax}$ (3.8 Hz) > $J_{1eq,2eq}$ (1.4 Hz).[13] Values of $J_{1eq,2ax}$ = 3.5 and $J_{1eq,2eq}$ = 1.5 Hz are recorded for methyl α-L-oleandroside.[14] In contrast to this trend, but with incomplete nmr information recorded in each case, Brimacombe and Portsmouth,[15] Hofheinz, Grisebach, and Friebolin,[16] and Arcamone, *et al.*,[9] report $J_{1eq,2ax}$ = $J_{1eq,2eq}$ = 3, $J_{1eq,2ax}$ = $J_{1eq,2eq}$ = 2.4, and $J_{1eq,2ax}$ = $J_{1eq,2eq}$ = 2.0 Hz, respectively, for methyl α-3-*O*-acetylchromoside D, methyl α-L-mycaroside, and methyl *N*,*O*-diacetyldaunosaminide. Thus, in *no* reported case is $J_{1eq,2ax}$ < $J_{1eq,2eq}$. Consistency with these data, then, requires the assignment of the H-2e signal of compound **3** to the higher field position. It must be emphasized, however, that the proper assignment of the geminal hydrogen signals is not a prerequisite for the correct interpretation of the remaining nmr signals, or the assignment of preferred conformation at each chiral center.

Of the low-field group of signals, H-1, H-3, H-4, and H-5, the last named appears at highest field, as expected. Irradiation of the H-5 signal at δ 3.98 leads to the collapse of the doublet of doublets centered at δ 4.57. The multiplet at slightly lower field, δ 4.66, is unaffected. Thus, the signals centered at δ 4.57 and 4.66 are assigned to H-4 and H-3, respectively. The doublet of doublets assigned to H-4 displays one large coupling constant, 9.5 Hz, characteristic of *vicinal* axial–axial splitting, and one smaller coupling constant, 3.5 Hz, consistent with *vicinal* axial–equatorial interaction. Since the axial nature of the C-3 acetamido group has already been established, these data require an equatorial acetoxy group at C-4 and an equatorial methyl group at C-5. Thus, compound **3** is methyl α-3-acetamido-4-*O*-acetyl-2,3,6-trideoxy-L-*ribo*-hexopyranose. It may be noted that the nature of the H-4 signal clearly distinguishes the L-ribo from the L-xylo configuration.

The nmr spectrum of **3** indicates a high degree of conformational homogeneity (conformation IC) in chloroform solution as expected of α anomer. This suggests the application of reported[9] chemical shift values for the acetyl methyl group (equatorial $OCOCH_3$, δ 2.00–2.09; axial $NHCOCH_3$, δ 2.00–2.09) in the assignment of conformation to the C-3 acetamido and C-4 acetoxy groups. Although there is a good correlation in the case of compound **3**, numerous papers[17] warn of the unreliability of conformational assignments made on this basis; therefore, the correlation is noted, but no strong significance is attached to it. The chemical shift values of the *O*-methyl and C-5 methyl signals also fall into reported[18] ranges (axial OCH_3, δ 3.36–3.46; equatorial C-5 CH_3, δ 1.16–1.24).

A final comment on the proposed higher field position of H-2eq signal relative to H-2ax is in order, as there appears to be no recorded example of such an occurrence[19,20] in a 2-deoxyhexopyranose. The proposal is at least qualitatively explicable in terms of current knowledge. The work of Lemieux and Stevens[21] suggests that the neighboring axial *N*-acetyl group at C-3 would act to deshield the axial hydrogen at C-2 and shield the equatorial hydrogen. There are likely other forces at work, but this factor could indeed influence the relative field positions of the C-2 hydrogens in the direction observed.

In conclusion, ristosamine, as derived from ristomycin A, is a new deoxy amino sugar of natural origin. The assignment of structure reveals that ristosamine differs from both vancosamine[11] (isolated from vancomycin) and daunosamine[9] (derived from daunomycin).

Experimental Section

Ristosamine (1). Ristomycin A (50 g) was hydrolyzed by refluxing for 4 hr in 500 ml of 0.5 *N* HCl. A precipitate which separated on cooling was removed by filtration, and the filtrate was neutralized to pH 6 by means of Dowex 2 (OH⁻) ion-exchange resin. The aglycone which precipitated after neutralization was collected and dried (21.10 g).

The neutral solution was slowly passed through a column of *ca.* 150 ml of Dowex 50 (NH₄⁺) ion-exchange resin. The column was washed with water until neutral, and ristosamine was then eluted with a 25% aqueous acetone solution containing 0.25 *N* ammonium hydroxide. Elution was continued until a positive reaction with ninhydrin was no longer detectable in the eluate. The combined fractions were concentrated to 8–10 ml under vacuum at 30–40° and then poured into 600 ml of absolute ethanol, whereupon ristosamine precipitated. The mixture was allowed to stand for a few hours in a refrigerator. The precipitate was collected by filtration and dried over concentrated sulfuric acid to yield 2.15 g of light-yellow, amorphous powder.

Ristosamine Picrate. Crude ristosamine (250 mg) was dissolved in 2.5 ml of absolute ethanol with gentle warming, and 7.0 ml of a saturated solution of alcoholic picric acid was added.

The resulting precipitate was collected by filtration and washed with small amounts of absolute ethanol and ether. The dried product amounted to 241 mg (38%). It was purified by dissolution in acetone and precipitation with petroleum ether, mp 118–119° dec.

Anal. Calcd for $C_6H_{13}O_3N \cdot HOC_6H_2(NO_2)_3$: C, 38.30; H, 4.28; N, 14.89. Found: 38.67; H, 4.28; N, 14.98.

Ristosamine Hydrochloride. Ristosamine picrate (250 mg) was dissolved in 10 ml of nitrobenzene and the solution was extracted with 15 ml of 1 *N* HCl in a separatory funnel. The aqueous phase was separated, extracted with ether (2 × 10 ml), clarified with decolorizing carbon, and evaporated at a temperature not exceeding 30–40°. On adding acetone to the resulting light yellow syrup, crystalline ristosamine hydrochloride separated, 83.6 mg (68%), $[\alpha]^{21}D$ 34.3° (*c* 0.57, H₂O).

Anal. Calcd for $C_6H_{13}O_3N \cdot HCl$: C, 39.25; H, 7.68; N, 7.62; Cl, 19.31. Found: C, 38.81; H, 7.77; N, 7.59; Cl, 19.36.

Qualitative Reactions of Ristosamine. Keller-Kiliani Reaction. With the modified procedure of Euw and Reichstein[22] ristosamine hydrochloride and daunosamine[9] exhibited an intense brownish-red color. Under similar conditions a blue color was obtained with digitoxose, and a greenish-blue color with 2-deoxy-D-ribose.

Xanthydrol Test. As with 2-deoxy-D-ribose and digitoxose, the xanthydrol test produced a reddish-violet color in the case of ristosamine hydrochloride. With glucosamine, galactosamine, and L-rhamnose, which were used as controls, no coloration was observed.

Iodoform Test. From 50 mg of ristosamine hydrochloride, 6 mg of iodoform was obtained, mp 119–120°, mmp 120–121°.

Oxidation of Ristosamine with Periodic Acid. Detection of Formaldehyde. After the oxidation of ristosamine with periodic acid, no formaldehyde was detected by the spectrophotometric method of Speck and Forist.[23] With glucosamine and galactosamine, the determination was reproducible.

Detection of Acetaldehyde. Ristosamine hydrochloride (150 mg) was dissolved in 61.00 ml of 0.05 *N* periodic acid and the mixture was allowed to stand at room temperature for 3 hr in the dark. Then the reaction mixture was distilled *in vacuo* (in a nitrogen at-

mosphere) into 20 ml of 3% 2,4-dinitrophenylhydrazine. The precipitate was filtered, washed with distilled water, dried *in vacuo* at 56° over phosphorus pentoxide, and recrystallized from *sec*-butyl alcohol to yield 52 mg of acetaldehyde 2,4-dinitrophenylhydrazone, mp 154–157°, mmp 152–156°. The 2,4-dinitrophenylhydrazone displayed a tlc R_f value identical with that of authentic acetaldehyde 2,4-dinitrophenylhydrazone on Kieselguhr G (nitrobenzene–cyclohexane, 1:2). The ir spectra (KBr) of both products were completely identical.

Methyl Ristosaminide Hydrochloride. Ristomycin A sulfate (21.0 g), dried to constant weight at room temperature over P₂O₅, was dissolved in 210 ml of 3 *N* methanolic hydrochloric acid and methanolyzed in a sealed ampoule at 105° for 5 hr. After cooling in an ice bath the ampoule was opened and its contents were evaporated at reduced pressure at 30° to the thickness of a syrup. The residue obtained after evaporation was dissolved in 50 ml of methanol and poured, at a slow rate, onto 170 ml of Dowex 1 (HCO₃⁻) ion-exchange resin suspended in 300 ml of distilled water. The precipitated aglycone was then removed from the solution by filtration followed by centrifugation. The pH of the solution was adjusted to 8.0 with 0.5 *N* ammonium hydroxide. The resulting precipitate was removed by filtration.

The filtrate containing the methyl ristosaminide was poured onto a Dowex 50 (NH₄⁺) ion-exchange column (30 × 4.5 cm). After washing with distilled water the column was eluted with 0.5 *N* ammonium hydroxide, collection being made in 200-ml fractions. The second fraction was acidified to pH 6.0 with a small amount of 3 *N* methanolic hydrochloric acid and evaporated *in vacuo* at 30° until methyl ristosaminide hydrochloride separated in crystalline form. Recrystallization from ethanol–acetone gave 350 mg (19%) of white, rodlike crystals, mp 168–170°, $[\alpha]^{20}D$ −123.8° (*c* 1, H₂O).

Anal. Calcd for $C_7H_{15}O_3 \cdot HCl$: C, 42.54; H, 8.15; N, 7.08; Cl, 17.93; OCH₃, 15.70. Found: C, 43.05; H, 8.05; N, 6.73; Cl, 17.43; OCH₃, 15.50.

One mole of **2** prepared as above consumed 0.70 mol of sodium periodate, measured with the procedure of Dixon and Lipkin.[6]

Methyl *N,O*-Diacetylristosaminide. Methyl ristosaminide hydrochloride (200 mg) was dissolved in the mixture of 2 ml of absolute pyridine and 2.0 ml of acetic anhydride and allowed to stand for 24 hr at room temperature. Then the reaction mixture was poured into 100 ml of ice water and extracted in three portions, each with 10 ml of chloroform. The combined organic phase was washed to neutrality with distilled water, dried over magnesium sulfate, and evaporated to dryness *in vacuo*. The residue was dried at room temperature in a vacuum desiccator over phosphorus pentoxide to constant weight, 179.6 mg of colorless oil.

Crude methyl *N,O*-diacetylristosaminide was dissolved in the mixture of 1.50 ml of absolute methanol–benzene (15:85), applied onto a silica gel G column (1.5 × 21 cm), and eluted with a solvent mixture of similar composition. Fractions were collected by 2-ml portions. The pure and homogeneous **3** obtained in the eighth fraction was recrystallized, after solvent removal *in vacuo*, from petroleum ether: 109.6 mg (40.1%) of white prisms; mp 51–52°; $[\alpha]^{21}D$ −134° (*c* 0.5, CHCl₃); thin layer chromatography R_f 0.31 [silica gel G, absolute methanol–benzene (15:85), sprayed with 50% sulfuric acid and developed at 120°]; nmr (220 MHz, CDCl₃) δ 1.22 (3 H, d, *J* = 7 Hz, C-6), 1.89 (1 H, broadened d of d, $J_{2eq,2ax} = 14$, $J_{2eq,1} <$ 1, $J_{2eq,3} = 2.5$ Hz, H-2eq), 2.00 (3 H, s, NHCOCH₃), 2.02 (3 H, s, OCOCH₃), 2.09 (1 H, d of t, $J_{2ax,2eq} = 14$, $J_{2ax,1} = 4$, $J_{2ax,3} = 3.5$ Hz, H-2ax), 3.43 (3 H, s, OCH₃), 3.98 (1 H, m, H-5), 4.57 (1 H, d of d, $J_{4,3} = 4.0$, $J_{4,5} = 9.5$ Hz, H-4), 4.66 (1 H, m, H-3), and 4.79 (1 H, d, $J_{1,2ax} = 4$, $J_{1,2eq} < 1$ Hz, H-1).

Anal. Calcd for $C_{11}H_{19}O_5N$: C, 53.87; H, 7.81; H, 5.71; OCH₃, 12.65; COCH₃, 35.10. Found: C, 54.27; H, 7.15; N, 5.75; OCH₃, 11.88; COCH₃, 35.85.

Methyl *N*-Benzoylristosaminide. Methyl ristosaminide hydrochloride (126 mg) and 143 mg of sodium carbonate were dissolved in 3.7 ml of distilled water and into this solution was dipped, under constant stirring, the solution of 0.083 ml of benzoyl chloride in 1.6 ml of absolute tetrahydrofuran. Stirring was continued for an additional 30 min. Checking with indicator paper showed that during this period of pH of the solution remained weakly alkaline. The reaction mixture was then diluted with 20 ml of distilled water and deionized with 5.0 ml of Dowex 2 (OH⁻) and 10 ml of Dowex 50 (H⁺) ion exchange resins. The resin was filtered and thoroughly washed with distilled water and the filtrate (pH 6.0) was extracted three times with 10 ml of ether. The combined ethereal phase was dried over magnesium sulfate and filtered and the solvent was evaporated *in vacuo*. The residual colorless oil was dried to constant weight in a vacuum desiccator: 126.0 mg (76.3%);

$[\alpha]^{20}$D $-104.3°$ (c 0.79, benzene); tlc R_f 0.55 [silica gel G, absolute methanol–benzene (15:85), sprayed with 50% sulfuric acid and developed at 120°].

Anal. Calcd for $C_{14}H_{19}O_4N$: C, 63.29; H, 7.22; N, 5.28. Found: C, 62.95; H, 7.20; N, 5.60.

N-Benzoylristosamine. Methyl *N*-benzoylristosaminide (111 mg) was hydrolyzed in 6.0 ml of 0.1 *N* hydrochloric acid for 1 hr on a steam bath. The cooled solution was neutralized to pH 7.0 with about 1.0 ml of Dowex 2 (OH⁻) resin, filtered, and evaporated to dryness. On standing in the refrigerator, the compound 5 crystallized as fleecy needles. Recrystallization from distilled water gave 53.9 mg (51.3%), mp 131–133°, $[\alpha]^{20}$D $-14°$ (c 1, ethanol), after 10 min $[\alpha]^{20}$D $-11°$, tlc R_f 0.25 (conditions described above).

Anal. Calcd for $C_{13}H_{17}O_4N$: C, 62.14; H, 6.81; N, 5.57. Found: C, 61.56; H, 6.69; N, 5.50.

N-Benzoyl-D-aspartic Acid from N-Benzoylristosamine. *N*-Benzoylristosamine (130 mg) and 135 mg of sodium periodate were dissolved in 4.0 ml of distilled water. The reaction mixture was then allowed to stand for 20 hr at room temperature in the darkness. In the meantime the pH of the mixture was maintained at 4–5 with 0.5 mol of sodium bicarbonate. At the end of the reaction time the solution was evaporated to dryness at a temperature not exceeding 30°. The residue was taken up with 5.0 ml of absolute ethanol and filtered and the solvent was removed. This latter procedure was repeated three times. The residual gum was dissolved in 11 ml of distilled water, and 1 g of calcium carbonate and 14 ml of aqueous bromine were added. The reaction mixture was allowed to stand for 20 hr at room temperature and then filtered. The excess bromine was expelled from the solution by sweeping with nitrogen gas. The mixture was agitated with 1 g of silver carbonate for 5 min and again filtered. The filtrate was acidified to a pH of 1–2 with Dowex 50 (H⁺) resin. The solvent was removed to yield a light yellow gum which crystallized from absolute ethanol, 12.0 mg (~10%). Recrystallization from distilled water gave mp 163–165° (lit.[24] mp 163–164°), $[\alpha]^{25}$D $-14.6°$ (c 1.28, H_2O containing 2 equiv of sodium hydroxide) [lit.[24] $[\alpha]^{23}$D $-22.3°$ (c 1.3, H_2O containing 2 equiv of potassium hydroxide)].

The R_f values of 0.34 and 0.76, respectively, on silica gel G tlc in solvent mixtures *n*-propyl alcohol–ammonium hydroxide (70:30) and *sec*-butylalcohol–formic acid–water (75:15:10) were in agreement with those obtained from the *N*-benzoyl-L-aspartic acid prepared by us. The ir spectra were likewise identical.

Registry No.—1, 51869-30-8; **1** picrate, 51869-31-9; **1** HCl, 51869-32-0; **2**, 51869-33-1; **2** HCl, 51869-34-2; **3**, 51869-35-3; **4**, 51869-36-4; **5**, 51869-37-5; **6**, 4915-59-7; ristomycin A, 11021-66-2; ristomycin A sulfate, 51932-11-7.

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Alkaloid Studies. LXVIII.[1] Novel Piperidyl Alkaloids from *Lupinus formosus*

William L. Fitch, Peter M. Dolinger, and Carl Djerassi*

Department of Chemistry, Stanford University, Stanford, California 94305

Three new alkaloids, (+)-*N'*-methylammodendrine (3), *N*-acetylhystrine (4), and the biogenetically intriguing smipine (9), have been isolated from *Lupinus formosus* and their structures determined. Other alkaloids identified were hystrine (1), (+)-ammodendrine (2), (−)-anabasine (5), (−)-*N*-methylanabasine (6), lupinine (7), and *N*-methylpelletierine (8).

In conjunction with an ecological study comparing the predation patterns and alkaloidal contents of several Colorado *Lupinus* species,[2] we undertook an investigation of the alkaloids of a local species, *L. formosus* Greene, collected within a few miles of the Stanford Chemistry Department.

The alkaloids were isolated and identified by standard techniques. The two major alkaloids were hystrine (1)[3] and (+)-ammodendrine (2).[4] To our knowledge this is the first report of the occurrence of these bipiperidyl alkaloids in a *Lupinus* species,[5] although they have been found together in the related legume genus *Genista*.[6] (+)-Ammodendrine and racemic ammodendrine have been encountered in several genera of the Leguminosae.[7]