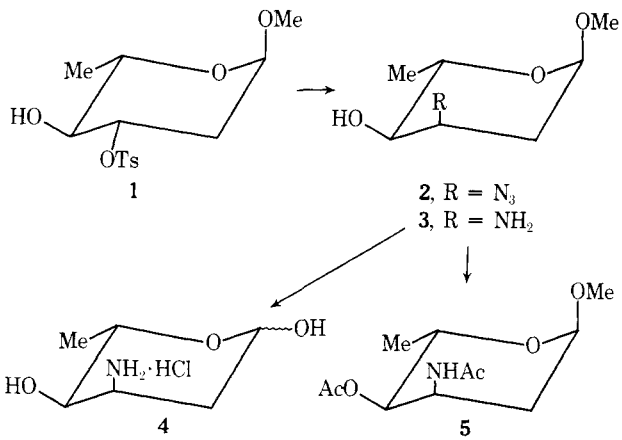
Communications to the Editor

Confirmation by Synthesis of Ristosamine as 3-Amino-2,3,6-trideoxy-L-*ribo*-hexose

Sir:

Several recently reported antibiotics have 3-amino-2,3,6-trideoxy-L-hexoses incorporated in their structures. The important antitumor agents, daunomycin[1b] and adriamycin,[1a] contain the sugar daunosamine, 3-amino-2,3,6-trideoxy-L-*lyxo*-hexose. Both variants of the antibiotic, ristomycin,[2] contain an amino sugar, ristosamine,[3] which has been assigned the structure 3-amino-2,3,6-trideoxy-L-*ribo*-hexose by Bognar et al.[4] on the basis of chemical and spectral properties. We have been interested in the synthesis of these novel amino sugars both from the standpoint of structure verification and in order to provide new sugars to couple with the aglycon from adriamycin in attempts to improve the antitumor activity of that antibiotic. In this manuscript we report the synthesis of 3-amino-2,3,6-trideoxy-L-*ribo*-hexose and confirmation of its identity with ristosamine and in the accompanying communication[5] report the synthesis of 3-amino-2,3,6-trideoxy-L-*arabino*-hexose and its identity with acosamine, a sugar derived from the antibiotic, actinoidin.[6]

A key intermediate in the daunosamine synthesis[7] is methyl 3-*O*-tosyl-2,3,6-trideoxy-α-L-*arabino*-hexopyranoside (**1**). Displacement of its tosyl group by azide ion in DMF at 120° for 18 hr afforded an 80% yield of the homogeneous azido alcohol **2**:[8] mp 78.5–80° (petroleum ether, bp 30–60°); $[\alpha]^{24}D$ −290° (*c* 0.5, $CHCl_3$); R_f 0.2 in $CHCl_3$ (R_f 0.4 for **1**).[9] Catalytic hydrogenation (5% Pd/C) in methanol at ambient temperature for 3 hr gave 85% of methyl 3-amino-2,3,6-trideoxy-α-L-*ribo*-hexopyranoside (**3**): mp 74–76° (benzene–hexane); $[\alpha]^{23}D$ −213° (*c* 0.5, $CHCl_3$); R_f 0.16 in MeOH–C_6H_6 (1:4) (R_f 0.35 for **2**). Treatment with 1.5 equiv of HCl in methanol precipitated **3**·HCl [mp 175–176° (methanol–acetone); $[\alpha]^{21}D$ −141.3 ± 1.5° (*c* 1.0, H_2O)] which compared well with methyl ristosaminide hydrochloride [mp 168–170°; $[\alpha]^{23}D$ −123.8° (*c* 1, H_2O)].[4]



Presumably the somewhat less negative rotation of methyl ristosaminide hydrochloride is because of its preparation involving acid treatment.[10] The hygroscopic **3**·HCl did not keep well. After 3 weeks in a desiccator, it had mp 159–160°, which could be raised to the original value by recrystallization (mp 176–177° from methanol–ether) but not by redrying.

Hydrolysis of **3** in 0.2 *N* HCl (95°, 90 min) followed by partial neutralization and lyophilization afforded the extremely hydroscopic, but solid 3-amino-2,3,6-trideoxy-L-*ribo*-hexose (analyzing for **4**·0.5H_2O): $[\alpha]^{21}D$ −42.2° (*c* 0.56, H_2O) at equilibrium; R_f 0.44 (R_f 0.38 for daunosamine hydrochloride) by paper chromatogram (Whatman No. 1) in butanol–acetic acid–water (5:2:3). An earlier sample prepared in 1966 had $[\alpha]D$ −26.9° (*c* 0.42, H_2O) at equilibrium, presumably because of differences in precautions against moisture during handling. The reported value[4] of $[\alpha]^{21}D$ +34.3° (*c* 0.57, H_2O)[11] for ristosamine hydrochloride falls between the above values.

Table I. NMR[a] of Synthetic and Natural **5**

Proton[b]	Chemical shift and multiplicity: Natural	Synthetic
H-6, 3	1.22, d[c]	1.20, d[c]
H-2_{eq}, 1	1.89, d of d[d]	1.90, d of br s[d]
NAc, 3	2.00, s	2.00, s
OAc, 3	2.02, s	2.02, s
H-2_{ax}, 1	2.09, d of t[e]	2.10, d of t[e]
OCH_3, 3	3.43, s	3.43, s
H-5, 1	3.98, m	3.92, m
H-4, 1	4.57, d of d[f]	4.50, d of d[f]
H-3, 1	4.66, m	4.58, m
H-1, 1	4.79, d of d	4.71, d of d

[a] Determined in $CDCl_3$ with internal Me_4Si; natural at 220 MHz;[4] synthetic at 100 MHz for δ values and 360 MHz for *J* values. [b] Assignment followed by number of protons. [c] $J_{5,6}$ is 6 Hz. [d] $J_{1,2eq}$, $J_{2ax,2eq}$, and $J_{2eq,3}$ are <1, 14, and 2.5 Hz, respectively, for natural (nat.) **5**;[4] nm, 14.5, nm for synthetic (syn.) **5**, where nm = not measurable (in the broad singlet, but are estimated to be like the reported values for nat. **5**). [e] $J_{1,2ax}$ and $J_{2ax,3}$ are 4 and 3.5 (nat.), respectively, and 4.5 and 4.5 (syn.). [f] $J_{3,4}$ and $J_{4,5}$ are 4 and 9.5 (nat.), respectively, and 4.5 and 10.0 (syn.).

Treatment of **3** with acetic anhydride in pyridine afforded 70% of methyl *N,O*-diacetyl-3-amino-2,3,6-trideoxy-α-L-*ribo*-hexopyranoside (**5**·0.5H_2O): mp 53–55° (petroleum ether, bp 60–110°); $[\alpha]^{21}D$ −130.8 ± 1.8° (*c* 0.50, $CHCl_3$); R_f 0.4 in methanol–benzene. In our hands, **5** could not be dried in vacuo without reverting to a syrup. It remained crystalline only when air-dried on a filter. This may account for its analysis as **5**·0.5H_2O for all three samples prepared on different occasions. The reported properties[4] of methyl *N,O*-diacetylristosaminide, [mp 51–52°; $[\alpha]^{21}D$ −134° (*c* 0.5, $CHCl_3$)] agreed well with those of **5**. Examination of the NMR data and MS data provided further confirmation of the identity of synthetic and natural **3**·HCl and **5**. Thus the NMR data in Table I show that natural and synthetic **5** are identical. They show the same multiplicity patterns and coupling constants within the range of experimental error, and they show the same relative positions for the proton signals. The slight differences in chemical shifts, increasing in the downfield direction (to δ 0.08) are probably of instrumental origin and do not affect the interpretation of the data. The mass spectral data (70 eV) of natural and synthetic **3**·HCl and **5** were compared and showed only the slight differences in relative abundance that may be expected of data obtained on different occasions and different instruments.[12] Recently, the mass spectra of methyl *N,O*-diacetyldaunosaminide and other derivatives have been reported.[13]

The synthetic 3-amino-2,3,6-trideoxy-L-*ribo*-hexose and its derivatives were obtained by a stereospecific route.

Since the above comparison proves the identity of the natural and synthetic ristosamine and derivatives, the structure of ristosamine as 3-amino-2,3,6-trideoxy-L-*ribo*-hexopyranose is confirmed.

Acknowledgment. This work was performed under Contract No. N01-CM-33742 of Drug Research and Development, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education and Welfare. The opinions expressed in this paper are those of the authors and not necessarily those of the NCI. We are indebted to Dr. Robert R. Engle, DRD, NCI, for providing the starting material **1** through Contract No. N01-CM23203 with Starks Associates, Inc.

References and Notes

(1) (a) F. Arcamone, G. Franceschi, and S. Penco, *Tetrahedron Lett.*, 1007 (1969); (b) F. Arcamone, G. Cassinelli, G. Franceschi, P. Orezzi, and R. Mondelli, *ibid.*, 3353 (1968).
(2) G. F. Gauze, E. S. Kudrina, R. A. Ukholina and G. V. Gavrilina, *Antibiotiki (Moscow)*, **8**, 387 (1963).
(3) N. N. Lomakina, L. A. Szpiridonova, R. Bognar, M. Puskás, and F. Sztaricskai, *Antibiotika (Moscow)*, **13**, 975 (1968).
(4) (a) R. Bognar, F. Sztaricskai, M. E. Munk, and J. Tamas, *J. Org. Chem.*, **39**, 2971 (1974). (b) After our communication was submitted for publication, F. Sztaricskai, I. Pelyras, R. Bognar, and Gy. Bujtas, *Tetrahedron Lett.*, 1111 (1975), reported the synthesis of *N*-benzoylristosamine in confirmation of their structure proof.
(5) W. W. Lee, H. Y. Wu, J. E. Christensen, L. Goodman, and D. W. Henry, *J. Med. Chem.*, following paper in this issue.
(6) F. Sztaricskai, N. N. Lomakina, I. A. Spiridonova, M. S. Frina, and M. Pushkash, *Antiobiotics*, **2**, 126 (1967).
(7) J. P. Marsh, Jr., C. W. Mosher, E. M. Acton, and L. Goodman, *Chem. Commun.*, 973 (1967).
(8) Analysis of all the new compounds gave values that agreed with calculated values within ±0.4% for C, H, and N and also Cl when present.
(9) TLC was run on silica gel plates and visualized by H_2SO_4 spray.
(10) Compare the case of natural and synthetic methyl *N,O*-diacetyl-α-daunosaminide. See ref 1b, footnote 9.
(11) Omission of the negative sign is almost certainly a typographical error.
(12) K. Biemann, "Mass Spectrometry", McGraw-Hill, New York, N.Y., 1962, p 45.
(13) A. Vigevani, B. Gioia, and G. Cassinelli, *Carbohydr. Res.*, **32**, 321 (1974).

William W. Lee,* Helen Y. Wu, John J. Marsh, Jr. Carol W. Mosher, Edward M. Acton, Leon Goodman David W. Henry

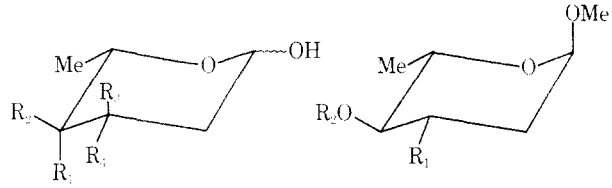
Bio-Organic Chemistry Department, Life Sciences Division Stanford Research Institute, Menlo Park, California 94025

Received March 24, 1975

Confirmation by Synthesis of the Structure of Acosamine and Methyl *N*-Acetylactinosaminide

Sir:

The accompanying communication[1] reports the synthesis of ristosamine, 3-amino-2,3,6-trideoxy-L-*ribo*-hexose, and the reasons for our interest in this family of amino sugars. In this manuscript we report the synthesis of 3-amino-2,3,6-trideoxy-L-*arabino*-hexose[2] and confirmation of its structural assignment to acosamine,[3] a component of the antibiotic, actinodin. Thus it is now feasible to replace the sugar moiety of adriamycin, daunosamine, with two other closely related sugars in efforts to modify the antitumor properties of adriamycin.[4] In addition, this manuscript describes the preparation of the methyl glycoside of 3-amino-4-*O*-methyl-2,3,6-trideoxy-L-*arabino*-hexose and confirmation of its assignment, by Lomakina et al.,[3] to methyl actinosaminide, a compound derived by methanolysis of actinoidin.



1. R_2 = OH; R_3 = NH_2
2. R_2 = OMe; R_3 = NH_2
3. R_1 = OH; R_3 = NH_2
4. R_2 = OH; R_3 = NH_2
other R's = H

5. R_1 = N_3; R_2 = H
6. R_1 = NH_2; R_2 = H
7. R_1 = NHAc; R_2 = H
8. R_1 = NHAc; R_2 = Ac
9. R_1 = NHAc; R_2 = Me
10. R_1 = NMeAc; R_2 = Me

Catalytic hydrogenation of methyl 3-azido-2,3,6-trideoxy-α-L-*arabino*-hexopyranoside (**5**), an intermediate in the synthesis of daunosamine,[5] afforded the amino **6**[6] in high yields with properties [mp 130–132° (benzene–hexane); $[\alpha]^{21}D$ −144° (*c* 0.52, MeOH); R_f 0.2 in C_6H_6–MeOH (4:1)][7] agreeing well with those previously reported[2] and less well with the rotation of methyl acosaminide, $[\alpha]^{20}D$ −118° (*c* 0.5, MeOH).[3,8] Heating **6** in 0.2 *N* HCl for 90 min at 90–95° followed by partial neutralization and lyophilization at pH 5.8 afforded high yields of the hydrochloride of 3-amino-2,3,6-trideoxy-L-*arabino*-hexopyranose,[6] analyzing for **1**·HCl·⅓H_2O, $[\alpha]^{21}D$ −18.3° (*c* 0.43, H_2O) at equilibrium.

Reaction of **6** with acetic anhydride in anhydrous methanol and in pyridine gave a quantitative yield of the *N*-acetyl **7** and 71% yield of the *N,O*-diacetyl **8**, respectively. Recrystallization from MeOH–ether afforded the analytically pure **7**[6] [mp 160–161°; $[\alpha]^{21}D$ −146° (*c* 0.52, MeOH); R_f 0.3 in benzene–methanol (4:1)] as compared to those reported for methyl *N*-acetylacosaminide [mp 161–162° (chloroform–ether); $[\alpha]^{20}D$ −90° (*c* 0.1, methanol)][3,8] and for the previously prepared **7**[2] [mp 159–160° (ether–petroleum ether); $[\alpha]^{20}D$ −148° (*c* 0.4, methanol)]. Recrystallization of **8** from ether–petroleum ether (bp 60–110°) yielded the analytically pure methyl *N,O*-diacetyl-3-amino-2,3,6-trideoxy-α-L-*arabino*-hexopyranoside (**8**):[6] mp 163–164°; $[\alpha]^{22}D$ −191° (*c* 0.52, MeOH). For methyl *N,O*-acetylacosaminide, the reported values[3] are mp 158–163° and $[\alpha]^{20}D$ −84° (*c* 0.5, MeOH); the rotation is again less negative.[8]

Methylation of **7** with dimethyl sulfate under the reported conditions[3] did not give the expected **9**, but two other products that mass spectral data suggested may be the dimethylated **10** and a dimeric compound. A different methylation procedure employing silver oxide and excess methyl iodide as solvent[11] afforded **9** [mp 188–190°; $[\alpha]^{21}D$ −150° (*c* 0.5%, MeOH)] as compared to methyl *N*-acetylactinosaminide (M) [reported mp 156–158° (ether–*n*-hexane), $[\alpha]^{20}D$ −70° (*c* 0.4, MeOH) when prepared from methyl *N*-acetylacosaminide; and mp 165–168° (chloroform–ether), $[\alpha]^{20}D$ −101° (*c* 0.6, MeOH) when prepared from methyl actinosaminide] but whose other properties (ir and NMR) were identical.[3] These reported results again may result from a difference in anomeric content[8] and, possibly, the presence of some dimethylated **10** in one case.

A comparison has been made of our NMR data and the data and figure reported for methyl *N*-acetylactinosaminide.[3] Its spectrum is reported to differ only by one methyl group[3] from that of methyl *N*-acetylacosaminide (details not reported except δ 3.30 for C_1-OMe). The data for **6–9** and M are tabulated in Table I. There is complete agreement between our results and the literature results. For all