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DETERMINATION OF THE STEREOCHEMISTRY AND ABSOLUTE CONFIGURATION AT C-4 AND C-5 OF 4-AMINO-4, 6-DIDEOXY HEXOSES

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Recently, 4-amino-4, 6-dideoxy hexoses have been isolated from both antibiotics and bacterial cell walls in increasing numbers (1). Consequently, the biological import and synthesis of these compounds have received increased attention (2). During the course of our own synthesis program (3), it became apparent that a straightforward, unequivocal method of general applicability for the determination of the stereochemistry and absolute configuration of C-4 and C-5 of 4-amino-4, 6-dideoxy hexoses was lacking. We now wish to report the development of such a method.

Previous degradation sequences (1c, 4) involved consecutive periodate oxidation, bromine-water oxidation and strong acid hydrolysis to a crude amino acid. The stereochemistry of the acid was established by paper chromatography comparisons with authentic samples. Then it was necessary to determine the absolute configuration of C-4 and C-5 by another set of reactions involving degradation of the methyl 4-amino-4, 6-dideoxy-α-hexoside to D'-methoxy-D-or L-methyl diglycolic aldehyde (4). In our hands, the degradation sequence of some of our methyl 4-acetamido-4, 6-hexosides to the amino acids led to undesirable side products which hampered a conclusive determination of stereochemistry by paper chromatography.

The degradation sequence which we propose involves a periodate oxidation of the 4-acetamido glycoside, followed by a sodium borohydride reduction of the resulting dialdehyde, an acid hydrolysis, and formation of a crystalline hydrogen oxalate salt. These reactions are homogeneous; the intermediates need not be purified. Thus, the stereochemistry and absolute configuration of C-4 and C-5 of the 4-acetamido glycoside can be established by unambiguous, classical organic chemistry such

as melting point and mixed melting point data, infrared spectra and optical rotation comparisons with authentic samples. Furthermore, if the identity of small quantities of aminosugar is required, this can be accomplished with 1-2 mg of amino alcohol hydrogen oxalate.

The scope of the degradation sequence is illustrated by the degradation of methyl 4-acetamido-4,6-dideoxy-α-D-gluco-(1d, 1f, 3a) and mannopyranoside (5) to D-allothreoninol hydrogen oxalate (D-A) and methyl 4-acetamido-4,6-dideoxy-α-D-galacto-(1e, 3b) and talopyranoside (3c, 6) to L-threoninol hydrogen oxalate (L-T). In detail, the methyl 4-acetamido-4,6-dideoxy hexoside is oxidized in 0.1 N sodium metaperiodate at room temperature for 4-6 hours. The resulting acetamido dialdehyde is reduced with a tenfold excess of sodium borohydride in water at 0° for 2 hours, then at room temperature for 12 hours. The N-acetyl group and glycoside linkage of the reduced product are cleaved with 1.5 N hydrochloric acid by heating on a steam bath for 12 hours. After purification via a Dowex 50 (H^+) chromatography eluting with methanol followed by 5% ammonia in methanol, the amino alcohol is converted to the crystalline hydrogen oxalate salt in ethanol. The yields of recrystallized amino alcohol salts range from 20-31% for the four steps. In each case, the identity of the oxalates was established by melting point, mixture melting point, superimposable infrared spectra (KBr) and specific rotation comparisons with authentic samples. The properties of the pure compounds, as well as those of various DL pairs, are given in Table I. It should be noted that the DL enantiomeric pairs melt higher than the pure enantiomers. The total melting point diagrams were determined and indicated solid solutions in both the allothreonine and threonine series. Thus, for example, a 1:1 mixture of D-T (mp $187-188^{\circ}$) and DL-T (mp $204-205^{\circ}$) has a mp $192-193^{\circ}$, and a 1:1 mixture of L-A (mp $174-175^{\circ}$) with D-T-L-A (mp $165-166^{\circ}$) has a mp $166-167^{\circ}$. Thus, it is important to interpret melting points and mixture melting points carefully.

For the biochemist or natural product chemist who does not have enough amino alcohol hydrogen oxalate for optical rotation measurements, melting point and mixture melting point data are unequivocal. For example, one melting point should place the oxalate

TABLE I

Compound [+]	Mp [*]	$[\alpha]_D$ in water [**]
D-T, L-T	187.5-188°, 188.5-189°	+7.15° (0.7), -7.84° (0.9)
DL-T	204-205°	
D-A, L-A	175-176°, 174-175°	-27.0° (0.6), +27.3° (0.7)
DL-A	201-202°	
D-A-L-T	165-166°	
D-A-D-T	200.5-201°	

[+] T = threoninol hydrogen oxalate; A = allothreoninol hydrogen oxalate.

[*] The melting points were corrected and taken on a Thomas-Hoover Melting Point Apparatus at 15°/min to within 15° of the melting point, then 5°/min to the melting point. All samples melted with decomposition. Melting points and rotations agree, where applicable, with literature values (7). [**] Concentrations listed in parentheses.

salt in the allothreoninol (mp 174-175°) or the threoninol (mp 187-188°) series. An infrared spectrum (KBr) compared to that of an authentic sample‡ should confirm this assignment since there are differences between T and A in the fingerprint region. Next, a 1:1 mixture melting point between the natural sample and either the D- or L-amino alcohol hydrogen oxalate in the same series will establish whether the unknown is D or L. If both samples have the same absolute configurations, the melting point will remain unchanged. If the samples are different enantiomers, the melting point will be elevated to that of the DL-mixture.

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‡ We have a limited supply of D and L-T and D and L-A available on request for chemists requiring samples for proof of structure. All four isomers were synthesized by known methods (7).

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