Configuration of the Ketosidic Bond of Sialic Acid*

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SUMMARY

The stereochemistry of the ketosidic bond of sialic acid has been elucidated by a study of the two anemic methyl ketosides. Isomer I was prepared by Koenigs-Knorr synthesis, and isomer II by direct methylation with methanol and an acid catalyst. These two anemic configurations were established by conversion with periodate-borohydride to III and IV, respectively, followed by lactonization of one isomer. Dreiding models demonstrate that only one anomer is potentially able to lactonize, and this was accordingly assigned Structure IV. This anomer and its precursor (II) were resistant to neuraminidase, whereas Compounds I and III were hydrolyzed by the enzyme. The configuration of the ketosidic bond of sialic acid in naturally occurring substances, such as gangliosides, glycoproteins, and the like, is therefore the less stable anomer I, with the ketosidic bond equatorial and the carboxyl group axial to the pyranoid ring. It is assigned the α-D configuration in accordance with the rules of nomenclature.

The sialic acids are a group of widely distributed amino sugars that occur in several glycoproteins, oligosaccharides, and glycolipids (1, 2). These acids are the naturally occurring derivatives of neuraminic acid in which the parent substance is acylated at the amino group with an acetyl or glycolyl moiety. The work of Comb and Roseman (3) showed that sialic acid can be enzymatically cleaved to N-acetylmannosamine and pyruvate and this served to establish the configuration of C-5 through C-8. Subsequently, Kuhn and Baschang (4) elucidated the stereochemistry at C-4 by unequivocal means. The only aspect of stereochemistry remaining to be determined is the configuration of the ketosidic bond joining sialic acid to the adjacent sugar. The two possible forms are shown in Fig. 1.

An earlier attempt to solve this problem utilized the change in optical rotation accompanying hydrolysis of the ketosidic bond of 3-O-N-acetylneuraminosyl-lactose by neuraminidase (5, 6). Since the change was negative this bond was assigned the α configuration in accordance with Hudson's isorotation rules (for D sugars). However, these rotational measurements at the sodium D line were small, and the anemic ketoside was not available for comparison. Two additional shortcomings of this approach have been discussed by Jeanloz (7): the possible influence of rotation of the carboxyl group attached to the anemic center, and the fact that the neuraminyl residue has the same general configuration as an L sugar. It may be noted in this connection that application of Hudson's lactone rule originally gave the wrong configurational assignment for C-4 (4).

Recently the two anemic methyl ketoses of sialic acid were synthesized by methods which closely resemble the classical reactions employed for preparing glycosides of the simpler sugars (10, 11). The product of the Koenigs-Knorr procedure was reported to be hydrolyzed by neuraminidase, while the anomer was not. However, the configurations were not established in these studies, and since it is not known to what extent the carboxyl group and other "special" features of sialic structure might influence the course of these reactions, it cannot be assumed a priori that the products possess the same stereospecificity as those derived from the simple sugars. Thus, Kuhn, Lutz, and MacDonald (10) noted that although their Koenigs-Knorr product was less levorotatory than the anomer, this observation could not be used to assign absolute configuration at carbon atom 2.

We have determined the anemic configurations of these two methyl ketoses by exploiting the differences in orientation of the carboxyl group relative to the hydroxyls, as reflected in lactone formation. The two methyl ketoses of N-acetylneuraminic acid were synthesized and subjected to the reactions shown in Fig. 2. Each was treated with sodium periodate to

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remove two carbons from the side chain, and following sodium borohydride reduction the resulting diol-acids were heated with \( \text{N}^+ \text{N}^- \text{dicyclohexylcarbodiimide} \) to determine which anomer could form a lactone. The diol-acid (IV) derived from the ketoside prepared by direct methylation of N-AN\(^2\), produced N, N'-dicyclohexylcarbodiimide to determine which anomer could form a lactone and therefore corresponded to Configuration II. The anomic diol-acid (III) derived from the ketoside prepared by Koenigs-Knorr synthesis did not lactonize and corresponded to Configuration I. Compound I \( (R = \text{CH}_3) \) was hydrolyzed by neuraminidase and thus represents the ketoside configuration of sialic acid in naturally occurring substances. In accordance with nomenclature rules, it is assigned the \( \alpha - \beta \) configuration.

**EXPERIMENTAL PROCEDURE AND RESULTS**

*Materials and Methods*

\( \text{N-Acetylneuraminic acid (type IV)} \) and \( \text{N-acetylneuraminosyl lactose (type I from bovine colostrum)} \) were obtained from Sigma, and neuraminidase enzyme \( (\text{Vibrio cholerae}) \) from General Biochemicals. Quantitative estimations of sialic acid and its derivatives were determined by the resorcinol method \( (12, 13) \), and free sialic acid by the thiobarbituric acid method \( (14) \). Dowex 50W-X8 (H\(^+\)) resin was used for deionization in some of the synthetic steps and as a catalyst for methylation. It was first recycled with sodium hydroxide and hydrochloric acid, thoroughly washed with methanol, and then dried in vacuum. In one preparation Amberlite MB-3 mixed-bed resin was used for deionization.

Thin layer chromatography was performed on glass plates \( (20 \times 20 \text{ cm}) \) coated with a 300-\( \mu \) layer of Silica Gel G. The latter was previously washed with chloroform-methanol \( (2:1) \) and acetone when used for preparative separations. \( \alpha \)-Butanol-acetic acid-water \( (100:40:40, \text{v/v}) \) was the developing solvent for all runs. Sialic acid and its derivatives were revealed by spraying with the resorcinol-HCl reagent, covering with a glass plate, and heating at \( 150^\circ \) for \( 20 \text{ min} \); in some instances charring with \( 50\% \text{ H}_2\text{SO}_4 \), was employed.

Gas-liquid chromatography was carried out with a F and M model 402 instrument equipped with dual flame ionization detectors and helium carrier gas. Sugars were chromatographed as the trimethylsilyl derivatives prepared according to the method of Sweeley et al. \( (15) \). The reaction was carried out in pyridine, and the solvent plus excess reagents were then evaporated under a stream of dry nitrogen. The residue was taken up in a small volume of hexane for injection. Four types of columns were used: \( (a) \) 3\% OV-1 on 100/120 Chromosorb W HP, \( (b) \) 3\% JXR on Gas-Chrom P, \( (c) \) 15\% EGSS-X on Gas-Chrom P, and \( (d) \) 3.8\% UC-W 84 on Diatoport S. The first was obtained from Supelco, Inc., Bellefonte, Pennsylvania, and \( b \) and \( c \) were from Applied Science Laboratories, Inc., State College, Pennsylvania, and \( d \) was a product of Union Carbide. All columns were 6 feet long, except the last which was 4 feet.

Mass spectra were obtained with an Hitachi-Perkin-Elmer RMU-6 instrument which had a direct inlet system. All spectra were recorded at 70 e.v. Infrared spectra were obtained with a Perkin-Elmer model 237 grating infrared spectrophotometer. Melting points were determined with a Kofler micro hot stage, and rotations with a Cary 60 spectrophotometer. Elemental analyses were performed by Schwarzkopf Microanalytical Laboratories, Woodside, New York.

**Synthetic Procedures**

*a. Methyl \( \alpha \)-Ketoside of N-AN-methyl Ester (Methyl Ester of I; \( R = \text{CH}_3 \)) — The Koenigs-Knorr procedure employed was similar to that of Kuhn et al. \( (10) \) with a few modifications. N-AN methyl ester, 181 mg, was prepared by stirring 200 mg of N-AN in 20 ml of absolute methanol with 1 g of Dowex 50 \( (\text{H}^+) \) at room temperature. It was at least \( 95\% \) homogeneous by GLC analysis and was not further purified. The pentaacetyl derivative of this ester was obtained as a colorless syrup, and the acetochloro compound as a white powder; each was used in the succeeding reaction without further purification. The acetochloro compound was converted smoothly to the methyl ketoside tetraacetate by stirring with methanol in the presence of silver carbonate, and the acetyl groups were then removed by mild base treatment in methanol. The final solution was deionized by passage through a Dowex 50 \( (\text{H}^+) \) column at \(-20^\circ \) in methanol solvent. Low temperature was found to be essential in this step to avoid partial isomerization of the anomeric center. The eluant was evaporated under a stream of nitrogen to give a colorless syrup which was dissolved in a small volume of ethyl ether-methanol \( (3:1, \text{v/v}) \). On standing in the cold, 56 mg of well formed crystals (prisms) were obtained, corresponding to \( 30\% \) yield based on N-AN-methyl ester. Melting point: 160-164°, \( [\alpha]_2^29 = -22.6^\circ \) (c, 0.21, in \( \text{H}_2\text{O} \)); reported value \( (10) \). -6.3° (c, 0.5, in methanol).

*b. The anomic ketoside II prepared by direct methylation (see Procedure b) was the more stable product and did not isomerize even when the deionization was performed at room temperature. In similar preparations of the methyl ketosides of L-sorbose (R. K. Yu and R. Ledeen, unpublished), we observed similar phenomena. The Koenigs-Knorr product completely isomerized on Dowex 50 \( (\text{H}^+) \) at room temperature, whereas the anomer was stable. At \(-20^\circ \) in methanol no isomerization occurred.

\[ \text{OH} \]
\[ \text{HOCH}_2 \]
\[ \text{CH} \]
\[ \text{NCNH} \]
\[ \text{COOH} \]
\[ \text{OH} \]
\[ \text{HOCH}_2 \]
\[ \text{CH} \]
\[ \text{NCNH} \]
\[ \text{COOH} \]

**Fig. 1. Structures of the two anomic ketosides of N-acetylneuraminic acid.**
Gas-liquid chromatography of the TMS derivative indicated approximately 95% purity, the OV-1 column was employed as it separated this compound from the anomic ketoside and from N-AN-methyl ester. This product gave the same color yield with resorcinol as N-AN (on a molar basis), but did not react with the thiobarbituric acid reagent. After conversion to the free acid the ketoside bond was hydrolyzed with neuraminidase (see below).

b. Methyl β-Ketoside of N-AN-methyl Ester (Methyl Ester of II; R = CH₃)—The procedure was similar to that of Kuhn et al. (10), except that a longer reaction time was employed. N-AN, 225 mg, was treated with 40 ml of absolute methanol and 0.5 g of Dowex 50 (H⁺) resin, and the mixture was refluxed with stirring for 48 hours. It was determined by a combination of resorcinol and thiobarbituric acid assays that 97% of the N-AN was converted to the methyl ketoside; after 24 hours only 85% had been transformed. The filtrate and washings from the resin were evaporated to give a yellow syrup which was dissolved in a small volume of ethyl ether-methanol (3:1, v/v). On standing in the cold, 145 mg of crystals (flakes) were deposited, corresponding to a yield of 60%. After one further crystallization the melting point was 111–115°, with some gas evolution at 90° (reported value (10): 115–130°), [α]D₂₀ = −45.4° (c, 0.13, in H₂O); reported value (10): −46° (c, 0.67, in methanol).

C₁₃H₂₈NO₇·H₂O
Calculated: C 46.39, H 6.87, N 4.15, OCH₃ 18.41
Found: C 45.98, H 6.60, N 3.89, OCH₃ 18.89

GLC analysis (EGSS-X column) of the TMS derivative indicated approximately 98% purity. This ketoside gave the same color yield with resorcinol as N-AN but did not react with the thiobarbituric acid reagent. Neither the ester nor the free acid was hydrolyzed by neuraminidase (see below).

c. Periodate-borohydride Treatment of I (R = CH₃)—In a typical run, 6.2 mg of the methyl ester of ketoside I (R = CH₃) were dissolved in 1 ml of methanol and treated with 1 ml of 0.2 M sodium metaperiodate in methanol-water (8:2, v/v). After standing 30 min at room temperature in the dark (some precipitate formed), 1 drop of ethylene glycol was added to destroy excess periodate. The resulting precipitate was removed by centrifugation and the cooled supernatant was treated...
with a solution of 25 mg of sodium borohydride in 7.5 ml of methanol. The mixture was stirred for 1 hour at 4°C and then deionized by passage through a column of Amberlite MB-3 at -20°C. Methanol was removed by evaporation and the resulting solid was treated with 1 ml of 0.05 N NaOH to neutralize the ester. This mixture was stirred for 3 hours at room temperature, then treated with 2 ml of acetone and passed through a Dowex 50 (II⁺) column at -20°C. Evaporation of the solvent gave 3 mg of amorphous white solid which could not be crystallized. TLC and GLC indicated the major product (III) to be approximately 90% pure.

d. Periodate borohydride Treatment of II (R = CH₃) Compound II (R = CH₃) was obtained from the corresponding methyl ester by mild alkaline hydrolysis. The ketoside ester, 68 mg, was dissolved in 5 ml of 0.06 N NaOH and allowed to stand for 2½ hours at room temperature. The resulting mixture was neutralized and deionized by passage through a column of Dowex 50 (H⁺) resin, and then lyophilized to give a white powder. It gave a single spot on TLC, corresponding to II (R = CH₃)

This powder was dissolved in 2 ml of H₂O and treated with 5 ml of 0.2 M sodium metaperiodate. The mixture was kept at room temperature in the dark for 1 hour with occasional shaking. Then 6 ml of 0.1 M barium acetate were added to precipitate iodate and periodate, and the resulting precipitate was filtered off in the cold. The filtrate was saturated with CO₂ to precipitate barium and the resulting mixture again filtered. The solution was reduced to 5 ml and treated with 40 mg of sodium borohydride, which was added slowly in the cold. After standing for 1 hour at room temperature the mixture was passed through a Dowex 50 (II⁺) column to deionize and to convert excess borohydride to baric acid. Lyophilization of the eluant gave a slightly yellowish solid, which was dissolved in a small volume of methanol, cooled, and evaporated under high vacuum. The methanol treatment was repeated five times to insure complete removal of boric acid. The resulting yellowish solid was crystallized twice from ethyl ether-methanol (4:1, v/v) to give 32 mg (60% yield) of IV as well formed white crystals. The melting point was 184–185°C, with some darkening at 170°C.

C₁₈H₂₁O₇N₃
Calculated: C 45.60, H 6.31, N 5.52, OCH₃ 11.79
Found: C 45.00, H 6.50, N 5.06, OCH₃ 13.38

A high degree of purity was indicated by the presence of a single spot on thin layer chromatography and a single peak on gas-liquid chromatography. The latter, the TMS derivative of the methyl ester was chromatographed on OV-1 and JXR columns.

e. Lactonization of IV—In a typical run, 7.2 mg of diol-acid IV was dissolved, together with 7.2 mg of N,N'-dicyclohexylcarbodiimide in 7 ml of redistilled pyridine, and the tube filled with nitrogen. The mixture was heated for 24 hours at 70°C, cooled, and spotted directly on a TLC plate. Three resorcinol-positive bands were visible: (a) Rp 0.25, corresponding to unreacted starting material; (b) Rp 0.64, the lactone fraction; and (c) Rp 0.75, N-acetyl-N,N'-dicyclohexylurea. These and other chromatographic constants are summarized in Table I. Only Band c was detected when a similar plate was sprayed with rhodamine 6G or water. Bands b and c were isolated by preparative TLC, the pyridine solution being concentrated by evaporation and spotted as a continuous streak on seven plates. After developing the plates and allowing the solvent to evaporate, water spray was employed to detect Band c. A narrow zone just below it corresponding to Band b was collected for the lactone even though the latter was not visible with water. A thin strip along the edge of the plate left unscraped was sprayed with resorcinol to confirm the location of the lactone. The silica gel scrapings were eluted with acetone to give 1.4 mg of lactone (V + VI) and 7.3 mg of N-acetyl-N,N'-dicyclohexylureas. The lactone product, after conversion to the TMS derivative, showed three peaks on GLC with the UC-W98 column. The major band comprising 74% of the total was isolated by preparative GLC and its structure established by infrared and mass spectroscopy (see below).

f. Attempted Lactonization of III—Diol-acid III was subjected to the same treatment as IV, e.g. heating with N,N'-dicyclohexylcarbodiimide in pyridine. TLC of the resulting mixture revealed two major resorcinol-positive bands: (a) a slow moving substance corresponding to starting material, and (b) a fast moving band which was also visible on spraying with water and rhodamine 6G. No bands were visible in the region expected for a lactone. The fast moving band was isolated by preparative TLC and shown to be acetylated N,N'-dicyclohexylurea (see below).

### Table I

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rp-TLC</th>
<th>Retention times—GLC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>JXR</td>
<td>OV-1</td>
</tr>
<tr>
<td>NαN</td>
<td>0.18</td>
<td>33.8 (195')</td>
</tr>
<tr>
<td>NαN-methyl ester</td>
<td>0.44</td>
<td>29.7 (195')</td>
</tr>
<tr>
<td>Methylα-ketoside of NαN-methyl ester (I)</td>
<td>0.46</td>
<td>23.8 (195')</td>
</tr>
<tr>
<td>NαN-methyl ester (II)</td>
<td>0.45</td>
<td>21.4 (180')</td>
</tr>
<tr>
<td>Diol-acid (III)</td>
<td>0.28</td>
<td>16.3 (180')</td>
</tr>
<tr>
<td>Diol-acid (IV)</td>
<td>0.57</td>
<td>11.0 (180')</td>
</tr>
<tr>
<td>Lactone V</td>
<td>0.64</td>
<td>11.2 (220')</td>
</tr>
<tr>
<td>Aocly NαN',N'-dicyclohexylureas of (III)</td>
<td>0.81</td>
<td>0.7 (220')</td>
</tr>
<tr>
<td>N-Acetyl-NαN',N'-dicyclohexylureas of (IV)</td>
<td>0.75</td>
<td>0.7 (220')</td>
</tr>
</tbody>
</table>

### Determination of Structures

**Lactone V**—The fractions isolated by preparative TLC (see Procedure e above) are shown on a chromatogram in Fig. 3. The lactone (Column 4) migrated ahead of the methyl ester of IV (Column 3), as expected for a product possessing one less hydroxyl group. Mild base treatment of the lactone regenerated...
the original acid as determined by TLC. The infrared spectrum of the lactone fraction revealed the following bands in the carbonyl region: 6.02 μ (strong) = amide; 5.74 μ (strong) = δ-lactone; 5.57 (weak) = γ-lactone. The latter band was believed to indicate the presence of a small proportion of γ-lactone (VI) rather than an anhydride since no species were detected on GLC in the region expected for such a dimer. This observation also provided evidence against an intermolecular ester, and the fact that the product emerged from the column with a shorter retention time than the methyl ester of the original acid (Table I) was also consistent with a lactone structure.

The most conclusive evidence for a lactone structure came from the mass spectrum of the TMS derivative (Fig. 4). The latter was purified by preparative GLC (see above) and corresponded to the major component of the lactone fraction (V). A number of the more significant ion fragments are listed in Table II along with the probable structures.

The parent ion was not observed, but a peak with m/e 302, corresponding to M-15 (loss of 1 CH₃ group), was present, which established the molecular weight. This phenomenon has been shown to be characteristic of TMS derivatives in general (16). The largest peak was m/e 73, corresponding to (Si(CH₃)₃)⁺, which has been frequently observed (16, 17) to be
the most abundant ion for TMS derivatives. The fragment with m/e 131 may correspond to an ion previously seen for the TMS of a neuraminic acid derivative (18). The prominent peak at m/e 84 is assigned on the basis of a similar peak detected in derivatives of 2 acetamido 2 deoxy sugars (10). The peaks at m/e 200, 175, 130, and 99 all indicate that the lactone formed with the primary rather than the secondary hydroxyl (i.e. a δ-lactone).

\[ \text{N-Acyl-N',N'-dicyclohexylurea from Dial-acid IV} \]

This side-product migrated on TLC slightly ahead of the lactone fraction (see Procedure e above and Fig. 3) and was the only band detected with rhodamine 6G and water sprays. The latter fact suggested a hydrophobic component in the structure. After isolation by TLC, it was analyzed by mass spectrometry and found to have a peak at m/e 467, corresponding to M-2. The presence of such a peak in place of the parent ion is frequently observed with alcohols (20). Prominent fragments with m/e values of 343, 218, and 126 gave strong support to the N-acylurea structure. Additional evidence was furnished by mild alkaline methanalysis and GLC identification of the products. These were found to be N,N'-dicyclohexylurea and the methyl ester of IV (chromatographed as the TMS derivative), which were present in approximately equimolar amounts.

\[ \text{Acyl N',N'-dicyclohexylurea from Dial-acid III} \]

This was the sole product obtained on attempted lactonization of III. It migrated on TLC slightly ahead of the corresponding product from IV (Table I), and was detectable with rhodamine 6G and water spray as well as resorcinol. After isolation by preparative TLC, its structure was determined by mild alkaline methanalysis and GLC identification of the products. The principal products were N,N'-dicyclohexylurea and methyl ester of III (chromatographed as the TMS derivative), which were present in approximately equimolar amounts. It was not established, however, whether this was the N-acetyl or O-acetyleneuraminosyl lactose was also treated under similar conditions and found to undergo 95% hydrolysis after 26 hours. The nature of the aglycone group thus appears to influence the rate of the enzyme. Compound III, derived from acid I (R = CH₃) was hydrolyzed by the enzyme whereas the anomer IV was inactive. These latter assays were carried out qualitatively by TLC.

**DISCUSSION**

The synthetic procedures outlined above for Compounds I and II (R = CH₃) and their methyl esters were patterned after previously described methods for these substances (10, 11, 21). Some modifications were introduced for the purpose of avoiding isomerization of the anomeric center in the case of I, and to increase the yield of methyl ketoside for II. The final products were at least 95% pure, according to GLC estimations, and the properties were in general agreement with the earlier findings. However, this appears to be the first report in which Compound I (R = CH₃, methyl ester) was obtained in crystalline form. Periodate-borohydride treatment of I and II gave diol-acids III and IV, respectively, but only diol-acid IV was obtained as crystalline material of high purity. Diol-acid III could not be crystallized and was estimated to be about 80% pure, but this was sufficient to establish the absence of lactone formation. Care was exercised in preparing these compounds to avoid conditions which would cause isomerization of the anomeric centers.

The reactions depicted in Fig. 2 provided an unequivocal assignment of the two anomeric configurations. Inspection of Drevilling models revealed that Structure IV is potentially able

**Table II**

<table>
<thead>
<tr>
<th>m/e</th>
<th>Probable Structure</th>
</tr>
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<tbody>
<tr>
<td>302</td>
<td>M - CH₃</td>
</tr>
<tr>
<td>274</td>
<td>M - CH₃CO</td>
</tr>
<tr>
<td>200</td>
<td>CH₃CHNCH₃</td>
</tr>
<tr>
<td>175</td>
<td>CH₃CHN(CH₂OSi(CH₃)₃</td>
</tr>
<tr>
<td>131</td>
<td>H₂NCH(OSi(CH₃)₃)</td>
</tr>
<tr>
<td>130</td>
<td>CH₂O</td>
</tr>
<tr>
<td>117</td>
<td>M - 200</td>
</tr>
<tr>
<td>99</td>
<td>130 - OCH₃</td>
</tr>
<tr>
<td>98</td>
<td>CH₃CHN(CH₂CH=CH₂)</td>
</tr>
<tr>
<td>84</td>
<td>CH₃CHNCH₃</td>
</tr>
<tr>
<td>73</td>
<td>Si(CH₃)₃</td>
</tr>
</tbody>
</table>
to lactonize in the boat (i.e. "flexible," "skew-boat") conformation with either one of the two hydroxyl groups. Structure III, on the other hand, with the carboxyl group trans to both hydroxyls, is not able to lactonize in any conformation because of the large distance always separating the groups. It was necessary to remove the 2 terminal carbon atoms from the side chains before attempting lactonization since the carboxyl group of I could conceivably react with the hydroxyl at C-9 to form an 8-membered lactone.

It was recognized that reaction of III and IV with N,N'-dicyclohexylcarbodiimide could give rise to additional products besides lactone, such as anhydrides, intermolecular esters, and substituted acylenes. The reactions were carried out in dilute solution in order to minimize the first two possibilities, and the conditions chosen were apparently successful in this regard since GLC retention times of the products revealed the absence of such dimers. The lactone fraction from IV isolated by TLC gave an infrared spectrum which indicated δ-lactone (V) to be the major product, with a small amount of γ-lactone (VI) also present. Prior to adopting this method of lactonization several other methods were attempted without success, such as refluxing in toluene or xylene with p-toluenesulfonic acid, treatment with HCl in methylene chloride, and so forth. The procedure which finally succeeded had the advantage of a mild basic solvent (pyridine), which would be expected to preclude isomerization at the anomeric center. The difficulty in forming lactone was possibly due to the necessity for the pyranoid ring to assume the less stable boat conformation, and this fact may also explain the low yield.

The major lactone was isolated in small amounts by GLC of the TMS derivative. Mass spectrometry provided clear proof that it was a δ-lactone possessing Structure V. The parent ion was not detected but the molecular weight was established from the m/e 302 fragment corresponding to M-15. As mentioned previously, the M-15 peak is characteristic of many TMS derivatives. Diol acid III gave no lactone, the only product being an acyl derivative of N,N'-dicyclohexylacrylurea. Such substituted acylenes were expected on the basis of the reaction mechanism (22), and a similar product was obtained from reaction of IV with N,N'-dicyclohexylcarbodiimide (see above).

These results now make it possible to assign Configuration I to the neuraminidase-susceptible anomer of N-AN ketoside. The only unverified assumption is that the enzyme has the same anomeric specificity with methyl ketoside as with the naturally occurring forms. This assumption appears sound in view of the wide variety of gangliosides, glycoproteins, and oligosaccharides which are hydrolyzed by the same enzyme, thus indicating a relative lack of specificity in regard to the aglycone. Our neuraminidase study did show a difference in ketoside rotations with those of sialic acid. The latter compound was suggested to have the anomic hydroxyl group axial to the pyranoid ring on the basis of nuclear magnetic resonance long range coupling constants, but the transposition of this finding to the ketoside structures depended on comparison of rotational measurements at the sodium D line. The shortcomings of this type of correlation in the case of sialic acid have been outlined above. These nuclear magnetic resonance studies did succeed in verifying the IC conformation for the pyranoid ring, however.

A by-product of the present study has been the demonstration that the reactions for synthesizing N-AN ketosides proceed with the same stereospecificity as for the simple glycosides. In both series the more stable anomer, with OCH₃ axial to the pyranoid ring, is produced by direct methylation with methanol and an acid catalyst, while the less stable anomer, with OCH₃ equatorial to the ring, is produced by the Koenigs-Knorr series of reactions.

Configuration I, corresponding to the neuraminidase susceptible anomer, is assigned the α-D configuration in accordance with the rules of nomenclature. This arises from the fact that C-7 with a β configuration is the configurational carbon, even though it is situated outside the ring. It is unfortunate that this aspect of the rules tends to obscure stereochemical relationships at the anomeric center, for it is evident that Structure III, which was derived from I without any change in pyranoid ring or ketoside configuration, must be given the opposite designation, β-L. This is because the configurational carbon atom is now C-6, according to the rules. Similarly, the β-D designation for Structure II changes to α-L in Structure IV. It should be noted that although the earlier application of Hudson’s rules gave the correct configurational assignment, this has turned out to be an “accident” of nomenclature. Since the ring form corresponds to an L sugar, the α anomer would be expected, on the basis of Hudson’s rules, to be more levorotatory, and this is opposite to what was observed. These nomenclature problems notwithstanding, the present study has established that the neuraminidase-susceptible ketoside of N-AN is the less stable anomer, with the carboxyl group axial and the ketoside bond equatorial to the pyranoid ring.

REFERENCES

9. The nomenclature of Lipids, Biochemistry, 6, 2387 (1967).

*Optical rotary dispersion studies of the methyl esters of I and II have shown that at low wave lengths the α anomer is decidedly more levorotatory, the crossover occurring at approximately 280 mp (R. K. Yu and R. Ledeen, unpublished observations).
Configuration of the Ketosidic Bond of Sialic Acid
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