THE SYNTHESIS OF ACETAMIDO-DEOXY KETOSES BY ACETOBACTER SUBOXYDANS. PART IV

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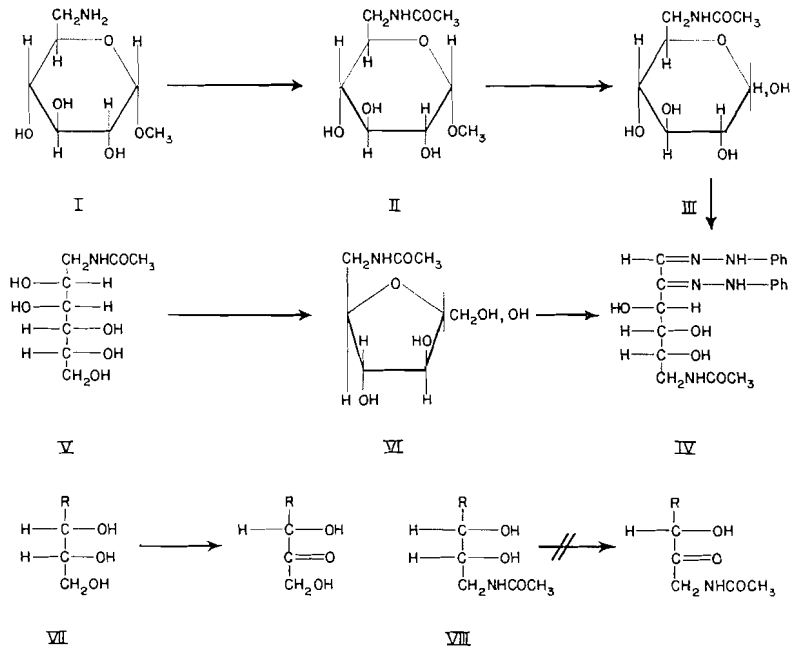
Methyl 6-amino-6-deoxy-α-D-glucoside (I) was prepared by the method of Cramer *et al.* (1) and *N*-acetylated with aqueous acetic anhydride (2) to give methyl 6-acetamido-6-deoxy-α-D-glucoside (II). Acid hydrolysis of the methyl glucoside (II) then gave 6-acetamido-6-deoxy-D-glucose (III) (1,3), from which a crystalline phenylosazone (IV) was prepared.



1-Acetamido-1-deoxy-D-mannitol (V) (4) was oxidized by *Acetobacter suboxydans* under conditions described in a previous publication (5) to give an 84% yield of a syrupy ketose sugar (VI). The ketose (VI) gave a crystalline phenylosazone (IV) which was identical with that obtained from 6-acetamido-6-deoxy-D-glucose (III). The ketose (VI) was therefore epimeric with 6-acetamido-6-deoxy-D-glucose (III) and thus possessed the D-fructose or D-*arabo*-hexulose configuration, and was therefore 6-acetamido-6-deoxy-D-*arabo*-hexulose.

As no other ketose was detected in the oxidation medium, it was concluded that oxidation of a polyhydric alcohol possessing the D-*erythro* configuration of hydroxyl groups according to the Bertrand–Hudson rules (VII) (6, 7) does not occur when the hydroxyl group of the primary alcohol group is replaced by an acetamido-deoxy group (VIII).

EXPERIMENTAL

Methyl 6-amino-6-deoxy-α-D-glucoside

Methyl 6-*O*-*p*-toluenesulphonyl-α-D-glucoside (5 g) (m.p. 116–118° C, $[\alpha]_D$ +106° (*c*, 2.2 in ethanol); lit. values: m.p. 124° C, $[\alpha]_D$ +98.5° (ethanol) (1)) was dissolved in absolute methanol (180 ml) and cooled

to 0° C. The solution was saturated with anhydrous ammonia, then heated in an autoclave at 120° C for 16 hours. After cooling, the dark solution was boiled with charcoal and filtered, and the filtrate was passed through Amberlite IRA 400 (OH^-) anion-exchange resin. The eluate was evaporated to dryness to give syrupy methyl 6-amino-6-deoxy-α-D-glucoside (3 g) (1).

Methyl 6-acetamido-6-deoxy-α-D-glucoside

Syrupy methyl 6-amino-6-deoxy-α-D-glucoside (3 g) was *N*-acetylated with aqueous acetic anhydride (2) to give crystalline methyl 6-acetamido-6-deoxy-α-D-glucoside. The product was recrystallized three times from methanol–ether to give needles (2.0 g) which had m.p. 163–164° C, $[\alpha]_D$ +133° (*c*, 2.0 in water) and gave absorptions in the infrared (potassium bromide disk) at 3500 cm^{-1} (OH), 3300 cm^{-1} (NH), 1640 cm^{-1} (amide I), and 1565 cm^{-1} (amide II).

Anal. Calc. for $C_9H_{17}O_6N$: C, 46.0%; H, 7.2%; N, 6.0%. Found: C, 46.0%; H, 7.7%; N, 6.2%.

6-Acetamido-6-deoxy-D-glucose

Methyl 6-acetamido-6-deoxy-α-D-glucoside (600 mg) was hydrolyzed with *N* sulphuric acid (15 ml) at 100° C for 3.5 hours. The solution was cooled to room temperature and passed through Duolite A4 (OH^-) anion-exchange resin and the eluate was evaporated to dryness. The residue was *N*-acetylated with aqueous acetic anhydride (2) as some de-*N*-acetylation had been observed in trial experiments under the same hydrolysis conditions. The product, which was obtained as a pale yellow syrup, contained unhydrolyzed methyl 6-acetamido-6-deoxy-α-D-glucoside and 6-acetamido-6-deoxy-D-glucose. The latter was separated by chromatography on Whatman 3MM paper using butan-1-ol–ethanol–water, 3:1:1, as solvent, and obtained as a clear, colorless syrup (100 mg), which crystallized on the addition of ethanol. When recrystallized from aqueous ethanol the 6-acetamido-6-deoxy-D-glucose (65 mg) had m.p. 203–205° C (decomp.), $[\alpha]_D$ +35±3° → +44±4° (18 hours) (*c*, 0.7 in water). (Lit. values: m.p. 182–183° C (decomp.), $[\alpha]_D$ +42° (in water, after 2 hours) (1) and m.p. 196–198° C (decomp.), $[\alpha]_D$ +44° → +35° (in water, after 24 hours) (3).)

6-Acetamido-6-deoxy-D-glucose gave absorptions in the infrared (potassium bromide disk) at 3300 cm^{-1} (OH and NH), 1625 cm^{-1} (amide I), and 1570 cm^{-1} (amide II).

6-Acetamido-6-deoxy-D-arabo-*hexose Phenylosazone*

The phenylosazone was prepared by the usual method using freshly distilled phenylhydrazine and glacial acetic acid. The phenylosazone was recrystallized from aqueous ethanol to give needles, m.p. 207–209° C (decomp.). The phenylosazone gave absorptions in the infrared (potassium bromide disk) at 3300 cm^{-1} (OH, NH) 3040 cm^{-1} (aromatic CH), 2900 cm^{-1} (aliphatic CH), 1635 cm^{-1} (amide I), 1580 cm^{-1} (amide II), and 1600, 1495, 745, 690 cm^{-1} (phenyl group).

6-Acetamido-6-deoxy-D-arabo-*hexulose*

1-Acetamido-1-deoxy-D-mannitol was oxidized by *Acetobacter suboxydans* under conditions described in a previous publication (5) and oxidation was complete after 21 days. The oxidation product was separated by chromatography on a cellulose column using butan-1-ol half-saturated with water as irrigant, and was obtained as a pale yellow syrup, $[\alpha]_D$ −8±2° (*c*, 1.94 in water). The ketose gave one spot on paper chromatography in several different solvent systems and gave a pink color with the orcinol – trichloroacetic acid spray reagent (8), changing to green on prolonged heating. The ketose gave absorptions in the infrared at 3300 cm^{-1} (OH, NH), 1725 cm^{-1} (saturated carbonyl group), 1640 cm^{-1} (amide I), and 1565 cm^{-1} (amide II). The infrared spectrum was obtained by smearing a little of the syrupy ketose on the surface of a potassium bromide disk.

The carbonyl peak at 1725 cm^{-1}, which was very weak, indicated that some of the ketose (ca. 5%) existed in the acyclic form and this was also suggested by the fact that the ketose slowly reduced Fehling's solution at room temperature (4).

6-Acetamido-6-deoxy-D-arabo-*hexose Phenylosazone*

The phenylosazone was prepared from the ketose under the same conditions as those used for 6-acetamido-6-deoxy-D-glucose. The phenylosazone was obtained as needles, m.p. 211–215° C (decomp.) and had a mixed melting point with the phenylosazone from 6-acetamido-6-deoxy-D-glucose of 206–209° C (decomp.). The infrared spectra of the two phenylosazones were identical over the range 4000–600 cm^{-1} and the two phenylosazones moved at identical rates on paper chromatograms in several different solvents.

Anal. Calc. for $C_{20}H_{25}O_4N_5$: C, 60.1%; H, 6.3%; N, 17.6%. Found: C, 60.3%; H, 6.9%; N, 17.5%.

Periodate Oxidation of 6-Acetamido-6-deoxy-D-glucose and 6-Acetamido-6-deoxy-D-arabo-*hexulose*

6-Acetamido-6-deoxy-D-glucose and 6-acetamido-6-deoxy-D-*arabo*-hexulose were each oxidized with an excess of sodium metaperiodate in unbuffered aqueous solution. The oxidation solutions were allowed to stand for 2 hours at room temperature, then tested for formaldehyde using the chromotropic acid method (9). 6-Acetamido-6-deoxy-D-glucose gave no formaldehyde, while 6-acetamido-6-deoxy-D-*arabo*-hexulose gave a strongly positive test for formaldehyde.

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HYPERFINE STRUCTURE IN THE ELECTRON SPIN RESONANCE SPECTRUM OF THE DIPHENYLENE RADICAL ANION

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The electron spin resonance spectra of the positive and negative radical ions of diphenylene were previously studied by McDowell and Rowlands (1). In that work it was mentioned that the observed five-line spectra could not be further resolved into the expected complete spectra of 25 lines. We now wish to report that we have been able to resolve completely the e.s.r. spectrum of the diphenylene radical anion. The radical anion was prepared by reacting the parent hydrocarbon with metallic potassium in purified, degassed, 1,2-dimethoxyethane as a solvent. The solvent had previously been carefully dried by allowing it to stand in contact with excess sodium anthracenide.

The spectra were recorded on a Varian e.s.r. spectrometer with 100 kc/s modulation and using a 12-in. Varian magnet. All the spectra were run at very low concentrations ($\sim 10^{-4}$ molar). The magnetic field calibrations were obtained by measuring the field with a proton resonance magnetometer, a 3-mm probe coil containing glycerol being inserted in the magnet gap. The associated marginal oscillator was frequency-modulated at 20 c/s and the proton resonance signal was displayed on an oscilloscope. A signal generator was loosely coupled and tuned for zero beat on the oscilloscope; the generator frequency and also the kystron frequency were measured with a Hewlett Packard 524/525/540 frequency counter which was standardized periodically against the WWV radio station.

Figure 1 shows the typical e.s.r. spectrum observed for the diphenylene radical anion in dimethoxyethane. The complete spectrum is shown in the upper portion of the figure while the detail of one of the five main bands is shown in the lower portion of the diagram. From the spectra we obtain the coupling constants for the two types of protons in diphenylene (see Fig. 2) to be $a^{H}_{(2)} = 2.76 \pm 0.01$ gauss, and $a^{H}_{(1)}$ to be 0.206 ± 0.008 gauss.

As is well known, the coupling constants for hyperfine splitting, a^{H}_{i}, due to protons are related to the average spin density, ρ_i, on the adjacent carbon atoms by the equation $a^{H}_{i} = Q\rho_i$. Q is a constant, negative in sign, which has been given various values such as