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Note

Synthesis of 2-amino-2-deoxy-D-glucose-^{15}N and of 2-amino-2-deoxy-L-glucose-2-^{14}C

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In the course of studies on the biosynthesis of the mitomycin antibiotics by *Streptomyces verticillatus*[1], it was observed that 2-amino-2-deoxy-D-glucose serves as an efficient antibiotic precursor. This result suggested that the nitrogen atom of the aziridine ring present in these antibiotics possibly originates from the amino group of this amino hexose. Nitrogen-15 labeled 2-amino-2-deoxy-D-glucose having a large atom-percent excess of the isotope was required for feeding experiments to test this hypothesis. 2-Amino-2-deoxy-L-glucose-2-^{14}C was also required for related biosynthetic studies.

Both syntheses followed closely the procedures used by Kuhn *et al.* for 2-amino-2-deoxy-L-glucose[2] and 2-amino-2-deoxy-D-glucose-1-^{14}C[3]. The starting material for the preparation of 2-amino-2-deoxy-D-glucose-^{15}N was ammonium-^{15}N nitrate, from which benzylamine-^{15}N was prepared by reaction with benzoyl chloride[4] with subsequent reduction by lithium aluminum hydride[5]. D-Arabinose was reacted with the amine to give *N*-benzyl-D-arabinosylamine-^{15}N, which without isolation was exposed for 2 h to a stream of hydrogen cyanide gas[6]. The resulting mixture of the nitriles of 2-benzylamino-2-deoxy-D-gluconic acid-^{15}N and 2-benzylamino-2-deoxy-D-mannonic acid-^{15}N, in which the former greatly preponderated[2], was converted by catalytic hydrogenation into 2-amino-2-deoxy-D-glucose-^{15}N hydrochloride (26% over-all yield) and a small amount of 2-amino-2-deoxy-D-mannose-^{15}N hydrochloride. 2-Amino-2-deoxy-L-glucose-2-^{14}C hydrochloride was prepared in 30% yield from L-arabinose-1-^{14}C, benzylamine, and hydrogen cyanide gas by analogous reactions. The purity of both compounds was ascertained by paper chromatography, and by optical-rotatory, mass-spectral, and radioactivity analyses where appropriate.

EXPERIMENTAL

General. — Ammonium-^{15}N nitrate having 95 atom-percent excess of ^{15}N was purchased from Stohler Isotope Chemicals Inc., and L-arabinose-1-^{14}C was obtained from Calbiochem. Mass-spectral analyses were performed in a Consolidated Electro-

dynamics Corp. Model 21-110 B high-resolution mass spectrometer with a direct-insertion probe. The M^+ and $M^+ + 1$ regions of ^{15}N-labeled samples and of the unlabeled reference compounds were scanned ten times and the relative ion-abundances were calculated. Radioactivity of solutions was measured in a Beckman Model LS 100 Liquid Scintillation spectrometer by using the scintillation solvent of Bray[7]. Radioactivity on chromatograms was detected with a Packard Model 7201 radiochromatogram scanner. Melting points were determined with a Thomas–Hoover apparatus and are not corrected. Optical rotations were measured with a Cary Model 60 spectropolarimeter.

Benzamide-^{15}N. — Ammonium-^{15}N nitrate (1 g) was dissolved in 6 ml of water, and 0.5 ml of benzene was layered on top of the solution, which was kept at 6°. Sodium hydroxide (1.14 g) dissolved in 5 ml of water and cooled to 6° was added slowly from a pipette, the tip of which was kept under the surface of the benzene layer. Benzoyl chloride (1.44 ml) in 50 ml of cooled benzene was then added quickly, and the stoppered flask was agitated vigorously with a magnetic stirrer for 2 h at room temperature. The mixture was then cooled and filtered. The aqueous phase was extracted five times with 10 ml of chloroform, and the organic phases were dried (sodium sulfate) and evaporated to afford 1.495 g of crystalline benzamide-^{15}N, m.p. 127°. From mass-spectral data (150°, 70 eV), the product was calculated to contain at least an 87 atom-percent excess of ^{15}N.

Benzylamine-^{15}N. — The benzamide-^{15}N so obtained was placed in a filter thimble that was inserted into a 150-ml cylindrical, pressure-equalizing dropping funnel to which a reflux condenser and a drying tube were attached. Lithium aluminum hydride (1 g) and absolute ether (250 ml) were refluxed for 4 h, allowing the ether to carry the amide slowly into the reaction medium. Remaining amide in the cup was removed with ether, and added to the hydride. The mixture was then stirred, for another 12 h, at room temperature. The reaction mixture was worked up by adding in sequence with stirring: 1 ml of water, 1 ml of 15% sodium hydroxide, and 3 ml of water[5]. This procedure afforded a readily filtered cake of aluminum hydroxide, which was washed with abs. ether. The combined ether solution was evaporated at low temperature to give benzylamine-^{15}N. Similar runs with unlabeled benzamide gave benzylamine in approximately 80% yield.

2-Benzylamino-^{15}N*-2-deoxy-*D*-glucononitrile.* — D-Arabinose (1.25 g), abs. ethanol (4 ml), and the total yield of benzylamine-^{15}N just described were refluxed gently with stirring for 15 min. The resulting, slightly yellow solution, presumably containing the glycosylamine, was transferred with 15 ml of abs. ethanol into a large test-tube. With appropriate precautions, a steady stream of hydrogen cyanide gas[6] was passed through the stirred solution. The crystalline nitrile began to form after 1 h, and the reaction appeared to be complete after 2 h. After cooling, the crystals were collected and air-dried; yield 1.804 g (55% based on benzamide), m.p. 131–132° (lit.[2] 130–132°).

A sample of the nitrile (23 mg) was converted with acetic anhydride in pyridine into the pentaacetate[2], and the product was recrystallized from ethanol (m.p. 129-

130°, lit.[2] 129–131°). Mass-spectral analysis (165°, 70 eV) showed the expected molecular-ions at *m/e* 476 and 477; their relative intensities were determined to be 5% and 95% respectively.

2-Amino-2-deoxy-D-*glucose*-^{15}N *hydrochloride.* — The foregoing nitrile (1.781 g) in 40 ml of 0.5M hydrochloric acid was hydrogenated at ambient temperature and pressure[8] in the presence of 180 mg of palladium chloride. Hydrogen consumption was 295 ml after 3 h. The slightly yellow solution was filtered and concentrated to give the amino sugar hydrochloride; yield 709 mg, $[\alpha]_D^{22}$ +69.2° (lit.[9] $[\alpha]_D$ +72.5°, final.) Descending paper-chromatography[10] revealed only one spot, which corresponded to authentic 2-amino-2-deoxy-D-glucose hydrochloride.

2-Amino-2-deoxy-L-*glucose*-2-^{14}C *hydrochloride.* — L-Arabinose-*1*-^{14}C (11 mmole, having a specific activity of 6.3 mCi/mmole), 100 mg of nonlabeled L-arabinose, 80 µl of benzylamine, and 5.5 ml of absolute ethanol were refluxed for 15 min. The reaction mixture was transferred to a small test-tube equipped with a magnetic stirring-bar. Gaseous hydrogen cyanide was bubbled through the mixture for 2 h, and 77 mg of 2-benzylamino-2-deoxy-L-glucononitrile-*2*-^{14}C, m.p. 130–131°, was obtained. The mother liquor afforded another 34 mg (62%) of the nitrile. The nitrile was dissolved in 40 ml of 0.5M hydrochloric acid and reduced with hydrogen gas after the addition of 10 mg of palladium chloride catalyst. Approximately 20 ml of hydrogen was consumed. After removal of the catalyst, the solution was concentrated to 0.2 ml and upon cooling 52 mg of crystals was obtained. Recrystallization from water–acetone afforded 43 mg (48%) of 2-amino-2-deoxy-L-glucose-*2*-^{14}C hydrochloride having a specific activity of 0.1 mCi/mmole. This material gave only one radioactive spot in paper chromatography[10] and radioactivity analysis, and also in t.l.c. on silica gel G with 80:20:10:3 methanol–butyl alcohol–water–conc. ammonia, (R_F 0.24). The optical purity of the product was ascertained by repeated co-recrystallization from water–acetone of 0.9 mg with 102 mg of the unlabeled D isomer. The specific radioactivity decreased from 8.54×10^3 d.p.m./mg (0.84 µCi/mmole) to 2.45×10^2 d.p.m./mg and 56 d.p.m./mg in the second and third crystallizations, respectively. Thus the product consisted of at least 99.4% of the L- isomer.

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