[CONTRIBUTION FROM THE DIVISION OF PLANT BIOCHEMISTRY, COLLEGE OF AGRICULTURE, UNIVERSITY OF CALIFORNIA]

Preparation of L-Arabinose-1-C^{14} [1]

BY D. A. RAPPOPORT AND W. Z. HASSID

The study of certain aspects of bacterial and plant pentose metabolism required the availability of L-arabinose-1-C^{14}. The preparation of this radioactive pentose was therefore undertaken.

By application of the Sowden–Fischer[2] nitromethane synthesis for ascending the aldose series of carbohydrates, Sowden[3] prepared D-glucose-1-C^{14} and D-mannose-1-C^{14}. The condensation of nitromethane with erythrose leads to the nitroalcohols which may subsequently be used to prepare D-erythro-2-desoxypentose.[4] It appeared possible that a similar method would be applicable for the synthesis of arabinose-1-C^{14} and ribose-1-C^{14}. Since we desired to synthesize the natural enantiomorph, L-arabinose-1-C^{14}, it was necessary first to prepare L-erythrose.

The earlier methods of preparing erythrose, depending on the degradation of arabinose[5] lead to impure sirups containing only small amounts of the tetrose. Sowden's method[4] of synthesizing D-erythrose by periodate oxidation of 4,6-benzylidene-D-glucose and subsequent hydrolysis, gave a product of good purity, but the yield of the benzylidene derivative was low. The method of Hockett, Collins and Scattergood[6] proved very satisfactory for the preparation of 4,6-ethylidene-L-glucose. Employing this procedure, we oxidized the ethylidene derivative with metaperiodate to 2,4-ethylidene-L-erythrose and upon hydrolysis obtained sirupy L-erythrose in good yield. The latter was condensed with nitromethane-C^{14} to yield a mixture of 1-nitro-1-desoxy-L-arabitol-1-C^{14} and 1-nitro-1-desoxy-L-ribitol-1-C^{14}. Condensation of erythrose with nitromethane resulted in good yields of the nitroalcohols, particularly when the ratio of erythrose to nitromethane was 3 to 1; the yield of nitroalcohols was 80% of theory. However, when the sodium nitroalcohols were treated with 18 *N* sulfuric acid in the Nef reaction[7] low yields from 2 to 10% of pentoses resulted.[8]

The mixture of L-arabinose-1-C^{14} and L-ribose-1-C^{14} was partially separated first by a cellulose column using butanol saturated with water as a developer.[9] The final separation of L-arabinose-1-C^{14} from L-ribose-1-C^{14} and from all radioactive impurities was made by means of partition chromatography on paper, using a phenol–water followed by a butanol–ethanol–water mixture. A single band of L-arabinose-1-C^{14} was thus obtained. Elution of the paper and concentration of the solution yielded a sirup of pure L-arabinose-1-C^{14}. The specific activity of this pentose was 1.2 μc/mg. In order to retain the high specific activity of the pentose, no attempt was made to crystallize the sirup by dilution with inactive crystalline L-arabinose.

Experimental

Preparation of 4,6-Ethylidene-L-glucose.—A quantity of 23.7 g. of L-glucose, prepared by the Sowden–Fischer procedure[2] was mixed in a glass stoppered bottle with 23.1 ml. of paraldehyde and 0.13 ml. of concentrated sulfuric acid. The mixture was shaken mechanically at room temperature for four hours. After standing at 28° for 72 hours, 100 ml. of ethyl acetate was added to the mixture, thoroughly mixed, and left in the refrigerator at 4° overnight. The crude crystals of ethylidene-L-glucose were filtered with suction and recrystallized three times from absolute ethanol containing a small amount of concentrated ammonia. The yield of 4,6-ethylidene-L-glucose was 13 g. (48%). Its specific rotation in water (*c*, 2) $[\alpha]_D$ was +2.2°; its melting point was 170–179°.

Anal. Calcd. for $C_8H_{14}O_6$: C, 46.6; H, 6.8. Found: C, 46.1; H, 6.7.

2,4-Ethylidene-L-erythrose.—To 11.68 g. of 4,6-ethylidene-L-glucose, 9.53 g. of powdered sodium bicarbonate was added and the mixture dissolved in an ice-cooled solution containing 24.2 g. of sodium metaperiodate in 334 ml. of water. The solution was allowed to stand for one hour at room temperature and subsequently concentrated to dryness under reduced pressure. The dry residue was extracted several times with warm ethyl acetate and after the latter was removed by distillation under reduced pressure, an amorphous white solid of 2,4-ethylidene-L-erythrose resulted; yield 7.7 g. (93%); m.p. 73–79°. Its specific rotation in water (*c*, 2.5) was $[\alpha]_D$ was +36°.

Anal. Calcd. for $C_6H_{10}O_4$: C, 49.3; H, 6.8. Found: C, 49.0; H, 6.8.

The product was analyzed for acetaldehyde by hydrolysis with sulfuric acid and steam distillation of the acetaldehyde into hydroxylamine–hydrochloride.[10] A 90% recovery of acetaldehyde, based on pure ethylidene erythrose, was obtained.

L-Erythrose.—Seven and one-half grams of ethylidene-L-erythrose was dissolved in 130 ml. of 0.1 *N* sulfuric acid and boiled under a reflux condenser for 90 minutes. The solution was cooled and deionized by means of Duolite A-4 and Amberlite IR-100. Concentration of the effluent under reduced pressure yielded 5.7 g. (92%) of a colorless sirupy L-erythrose. Its specific rotation in water (*c* 2) was $[\alpha]_D$ +20°.

1-Nitro-1-desoxypentitol-1-C^{14}.—The condensation of D-erythrose with nitromethane was first tested with inactive nitromethane, using varying proportions of the tetrose and sodium methylate. In all tests the resulting sodium nitropentitols were filtered, washed with methanol–ether (1:1), ether and petroleum–ether and then dried *in vacuo*. It was observed that the highest yield of nitroalcohols was obtained with ratios of erythrose to nitromethane to sodium methylate of 3:1:1.6, respectively. This ratio of reactants was used for the condensation of radioactive nitromethane with L-erythrose.

To a solution of 3.60 g. (30 m*M*) of L-erythrose in 10 ml.

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(2) J. C. Sowden and H. O. L. Fischer, THIS JOURNAL, **69**, 1963 (1947).

(3) J. C. Sowden, *J. Biol. Chem.*, **180**, 55 (1949).

(4) J. C. Sowden, THIS JOURNAL, **72**, 808 (1950); see also W. G. Overend, M. Stacey and L. F. Wiggins, *J. Chem. Soc.*, 1363 (1949).

(5) A. Wohl, *Ber.*, **32**, 3667 (1899); O. Ruff, *ibid.*, **32**, 3672 (1899); V. Deulofeu and R. Selva, *J. Chem. Soc.*, 225 (1929).

(6) R. C. Hockett, D. V. Collins and A. Scattergood, THIS JOURNAL, **73**, 599 (1951).

(7) J. U. Nef, *Ann.*, **280**, 263 (1894).

(8) In subsequent experiments, when hydrolysis of the nitroalcohols (Nef reaction) was conducted at 25 to 30°, a yield of pentose of approximately 30% was obtained.

(9) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, 2511 (1949).

(10) M. A. Joslyn and C. L. Comar, *Ind. Eng. Chem., Anal. Ed.*, **10**, 364 (1938); also see staff of Hopkins and Williams Research Laboratory, Organic Reagents for Organic Analysis, Chemical Publishing Company, 1946.

of absolute methanol was added a cooled solution of 0.368 g. (16 m*M*) of sodium metal dissolved in 10 ml. of absolute methanol. The flask with this mixture was attached to a gas manifold to which another flask was connected containing 6.1 g. (10 m*M*) of frozen nitromethane-C^{14} of approximately 1 mC. radioactivity. The nitromethane was then allowed to distill into the Dry Ice-cooled erythrose mixture. When the distillation was completed, the flask with the reaction mixture was detached, stoppered and allowed to stand at room temperature for 20 hours. The flask with the contents was cooled in an ice-bath, and an equal volume of ether was added to the mixture. The precipitated nitropentitols were rapidly filtered, washed with ether and petroleum ether and dried in a vacuum desiccator. The yield of sodium nitropentitols was 3.2 g. (77% based on the amount of nitromethane-C^{14} used).

Pentose-1-C^{14}.—A quantity of 9.0 ml. of 18 *N* sulfuric acid was introduced into a 50-ml. wide-mouth flask equipped with a magnetic stirrer, and placed in an isopropyl alcohol-Dry Ice-bath maintained at −10 to −15°. The solid sodium nitroalcohols (3.2 g.) were dissolved in a minimum amount of ice-cooled water and the solution added dropwise with stirring to the cooled sulfuric acid. The mixture was then allowed to stand with stirring for 15 minutes, 100 ml. of ice-water was added and the solution immediately passed through Amberlite IR-100 and Duolite A-4 columns. Each column was washed until the radioactivity in the effluent approached background counts. After concentration of the solution by vacuum distillation, 2.2 g. of crude pentose sirup was obtained.

Isolation of L-Arabinose-1-C^{14}.—The crude pentose sirup was dissolved in a minimum amount of water and the solution placed on a powdered cellulose (Whatman No. 1, ashless pellets) column, 22.5 inches long and 0.75 inch in diameter.[9] This column was attached to a "Technicon" fractionator and the sirup was fractionated, using butanol saturated with water containing approximately 0.3% concentrated ammonia. The fractions were collected in 400 test-tubes, each tube containing 1.2 ml. of eluate. The locations of L-arabinose and L-ribose were determined by paper chromatography of every tenth test-tube, using aniline phthalate as a pentose spray reagent.[11] The arabinose containing fractions were combined and a sample was chromatographed in two dimensions on Whatman No. 1 paper, first with butanol–ethanol–water and then with phenol–water. The chromatogram showed that in addition to the L-arabinose-1-C^{14}, a small amount of L-ribose-1-C^{14}, some inactive glucose and traces of two unidentified radioactive compounds were present.

Purification of L-arabinose-1-C^{14} was accomplished by means of band chromatography on paper. Of the partially purified sirup, 29 mg. was dissolved in 0.6 ml. of water and 0.25 ml. was deposited in 0.01-ml. portions along a penciled line on two sheets of Whatman No. 1 paper (22 × 18 inches). Inactive L-arabinose, placed along the edges of the paper sheets, was used as a marker. The papers were chromatographed for 24 hours with phenol saturated with water by the descending unidimensional technique. The papers were then dried, and radioautographs were made on an X-ray film. The arabinose band was identified by spraying the markers after they had been cut from the paper. The L-arabinose-1-C^{14} bands were then cut from the papers using the radioautographs as a guide, and then eluted with water. The solutions were combined and concentrated to dryness in a vacuum oven at 40°. The residue was dissolved in 0.3 ml. of water and chromatographed again on one sheet of paper as previously described using butanol–ethanol–water (21:13:5). Subsequently, the single band of L-arabinose-1-C^{14} was cut out, eluted with water and concentrated under reduced pressure to dryness. The yield was 1.2 mg. of L-arabinose-1-C^{14} with a specific activity of 2.2 × 10^5 counts/minute mg., which is equivalent to 1.2 μc/mg. The total yield of the synthetic L-arabinose-1-C^{14} was 22.2 mg. (3%, based on nitromethane-C^{14} activity).

The crude L-ribose-1-C^{14} can be similarly purified by the paper chromatographic procedure.

D-Arabinophenylosazone.—In order to further establish the identity of the labeled pentose material, D-erythrose was combined with inactive nitromethane as previously described. Upon treatment of the sirupy product with phenylhydrazine hydrochloride and sodium acetate,[12] a phenylosazone was obtained which was identified as that of arabinose; m.p. 160°.

Anal. Calcd. for $C_5H_8O_3(N_2H\cdot C_6H_5)$: C, 62.2; H, 6.1; N, 17.1. Found: C, 61.8; H, 6.1; N, 16.9.

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(11) S. M. Partridge, "Partition Chromatography," Biochemical Symposia No. 3, Cambridge University Press. 1950, p. 57.

(12) W. Z. Hassid and R. M. McCready, *Ind. Eng. Chem., Anal. Ed.*, **14**, 683 (1942).

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[CONTRIBUTION FROM ABBOTT LABORATORIES]

Local Anesthetics. II. Some Aryloxyalkyl Alkamine Ethers[1]

BY HOWARD B. WRIGHT AND M. B. MOORE

Paper I of this series reported the synthesis of various aryl alkamine ethers. The work is now extended to include related compounds in which the alkylene chain is interrupted by an oxygen, according to the general formula Ar–O–R′–O–R″–NR_2, in which Ar is an aryl or arylalkyl residue, R′ and R″ are bivalent alkylene radicals and NR_2 is the residue from a tertiary amine. The salts of these bases have been studied as local anesthetics.

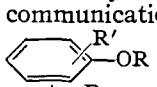
The aryl alkamine ethers reported in the previous communication[2] were of the general formula  (R′ = hydrocarbon or ether substituents, R = various alkamine residues) and were of sufficient pharmacological interest to indicate the desirability of studying other closely related compounds. Interruption of the alkylene chain by an oxygen seemed worthwhile and such compounds are reported in this paper. Some compounds of this type have been briefly described[3] but their therapeutic use was not suggested.

Table I lists the ethers synthesized, with pertinent physical and analytical data. Hydrochlorides of these compounds have been tested for local anesthetic activity by Dr. R. K. Richards and Miss Eunice Siewert, and all exhibited some degree of local anesthetic activity. Several of the salts, as those of the first two compounds in the table, resemble procaine in their local anesthetic action in wheals. Some, as in the case of the third compound in the table, produce good corneal anesthesia.

(1) Presented at the Division of Medicinal Chemistry, American Chemical Society, Cleveland, Ohio, April 8–12, 1951.

(2) H. B. Wright and M. B. Moore, THIS JOURNAL, **73**, 2281 (1951).

(3) H. A. Bruson, U. S. Patent 2,115,250, April 26, 1938.