RAPID SYNTHESIS OF 2-DEOXY-D (1-^{14}C) GLUCOSE SUITABLE FOR LABELLING WITH ^{11}C.

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SUMMARY

D-arabitol (I) treated with acetone gave 2,3-4,5 and 1,2-4,5 di-O-isopropylidene-D-arabitol (II) as shown by 13C NMR. This mixture was converted into the corresponding mixture of mesylates from which 2,3-4,5-di-O-isopropylidene-1-O-methane sulfonyl-D-arabitol (III) was isolated. The latter was converted into 1-deoxy-2,3-4,5-di-O-isopropylidene-1-iodo-D-arabitol (IV). (IV) and sodium (^{14}C) cyanide gave 2-deoxy-3,4-5,6-di-O-isopropylidene-D-(1-^{14}C)-glucononitrile (V) which was hemihydrogenated into 2-deoxy-D-(1-14C)-glucose (VI). The overall yield based on sodium-(14C)-cyanide was 30 % and the radiochemical purity was 98 %. The overall time of synthesis was about one hour. This is suitable for working with 20 minutes half-lived 11C.

The usefulness of 2-deoxy-D-(1-^{14}C)-glucose for the measurement of the local brain glucose metabolism in animals has recently been demonstrated by SOKOLOFF et al. (1). To extend this method to an "in vivo" study in man, the 2-deoxy-D-glucose has to be labelled with a gamma emitter. For this purpose, the fluorinated analog 2-deoxy-2-fluoro-D-glucose has been labelled with ^{18}F by IDO et al. (2). Although it seems that the metabolism of this compound is comparable to the non fluorinated one (3), a labelling without modifying the structure of this sugar would be preferable.

For this reason, we have attempted to develop a method adapted to the incorporation of the 20 minutes half-lived ^{11}C in 2-deoxy-D-glucose. Because of the short time available for such a

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synthesis, a route with the smallest number of radioactive steps was studied. Furthermore special care was taken to improve the chemical yield and to speed up the purification procedures. For these purposes, ^{14}C was used as a tracer.

Several syntheses of 2-deoxy-D-glucose have already been published. D-glucose has been converted through D-glucal and several steps into 2-deoxy-D-glucose (4,5,6,7,8,9,10). Sowden - Fischer's procedure applied to D-arabinose and nitromethane via the 1-nitro-1-deoxyalditol would involve 8 steps from $^{11}CO_2$ (11,12, 13). More recently, the Wittig reaction has been used with unprotected aldoses or protected aldoses and various ylides : phenylthio methylene (14) phenoxymethylene (15) methylthiomethylene (16) methoxymethylene (17) - triphenylphosphorane. 2-deoxy-D glucose has also been obtained by reduction of 2-deoxyaldonic esters (18).

None of the above procedures appears to be adaptable to ^{11}C labelling because of the too many steps involved.

The method we report here, outlined in scheme I, is an adaptation of that used by BAYLY and TURNER for the synthesis of 2-deoxy-D (1-^{14}C) ribose (19,20).

2,3-4,5-di-O-isopropylidene-D-arabitol (II) was prepared from D-arabitol (I) by the method described by BUKHARI et al. (21) and NAKAGAWA et al. (22). ^{13}C-NMR spectra have shown that a mixture of two isomers was obtained : both compounds had very similar ^{13}C-NMR spectra, each one presenting four methyl and two quaternary isopropylidene carbons, one secondary carbon and four tertiary carbons (determination by chemical shifts and off - resonance methods). The less important compound had the most symmetrical spectrum. The evaluation of chemical shifs with use of increments due to arabitol substituents has led to the conclusion that two isopropylidene 1,2-4,5 and 2,3-4,5 isomers were present. They were not isolated at this step but at the next one.

The esterification of the primary alcohol at C-1 with methane sulfonic anhydride (23,24) gave 2,3-4,5-di-O-isopropylidene 1-O-methanesulfonyl-D-arabitol (III) which was isolated by preparative chromatography.

This methanesulfonyl ester was converted into 1-deoxy-2,3-4,5-di-O-isopropylidene-1-iodo-D-arabitol (IV) by nucleophilic displacement with sodium iodide (19) in anhydrous methylethylketone at reflux temperature. The iodocompound (IV) reacted with sodium (^{14}C) cyanide in anhydrous dimethylformamide (19) to give 2-deoxy 3,4-5,6-di-O-isopropylidene-D-(1-^{14}C) gluconononitrile (V) with a yield of 75 to 80 % based on cyanide. This nitrile was converted

into 2-deoxy-D-(1-^{14}C) glucose (VI) by hemihydrogenation (25) using Palladium catalyst in dilute hydrochloric acid with the simultaneous removal of the di-O-isopropylidene groups. The use of Palladium black, a mild catalyst, avoided overreduction of the nitrile group to the amino group (20). The yield of this last step was 40 to 50 % based on nitrile and about 30 % based on cyanide. The radiochemical purity of (VI) was 98 %.

In summary, the synthesis of 2-deoxy-D-(1-^{14}C)-glucose here described was carried out with two active steps of respectively two minutes (nitrile) and ten minutes (reduction) and with a purification of five minutes by high performance liquid chromatography (HPLC). The first attempts to further reduce the duration of other chemical operations (extraction, evaporation, neutralization) showed that all the steps can be carried out within one hour, that is the limit of time allowed by the ^{11}C nucleus half-life. (see Scheme I).

EXPERIMENTAL

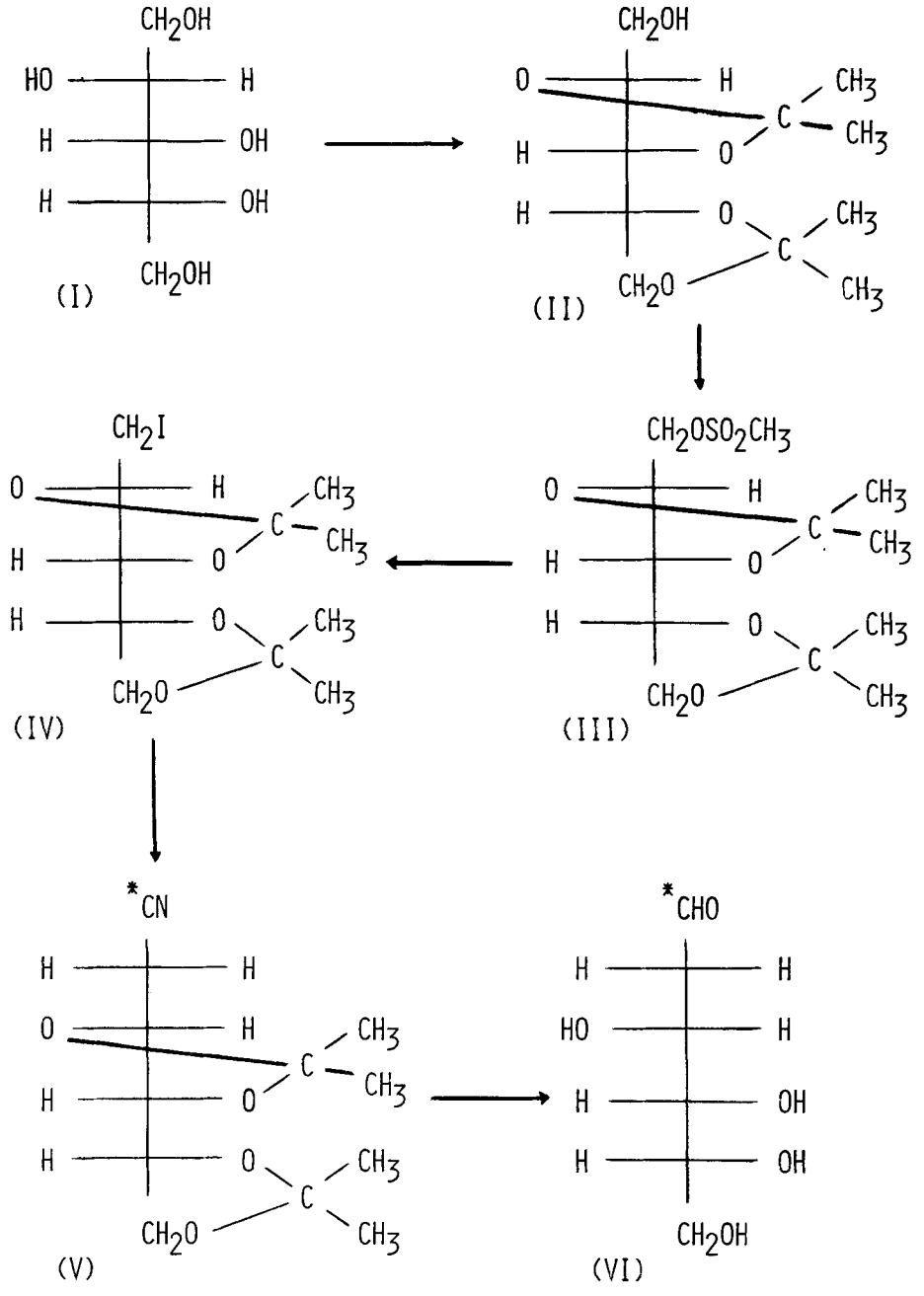
The different stages of the synthesis were checked by chromatographic methods (TLC,VPC and HPLC). The identity of all prepared products was confirmed by ^{13}C-NMR using a VARIAN CFT 20 spectrometer at 20 MHz and by mass spectrometry using a VARIAN CH 7A spectrometer.

Thin-layer chromatography (TLC) was carried out using MERCK silicagel 60 F 254 glass plates and a 50 % sulfuric acid solution as spray reagent for the II, III, IV, and V steps. SCHLEICHER and SCHULL F 1440 LS 254 cellulose sheets and a 1 % anisidine hydrochloride solution as spray reagent were used for step VI. Radioactivity on chromatograms was detected with a "CHROMELEC 101" radiochromatogram reader. The separation of VI in the ultimate step was carried out by HPLC using the WATERS ASSOCIATES chromatograph equipped with a refractive index detector and a PARTISIL PXS 10/25 PAC WHATMAN column eluting with Acetonitrile : Water (80 : 20) at 1.5 ml/min. ^{13}C-NMR chemical shifts were measured from the proton decoupled spectra with Benzene at 128.68 ppm from TMS. The spectra with proton coupling were taken with off-resonance. Usually 16 K data points, spectral width of 4500 Hz, 30° pulse (10 s) and 20000 pulses were used.

2,3-4,5-di-O-ISOPROPYLIDENE-D-ARABITOL (II)

was prepared from D-arabitol (I) (J.T. BAKER) according to NAKAGAWA et al. (22).

Scheme I



SYNTHESIS OF 2-DEOXY-D-(1-^{14}C)-GLUCOSE

TLC was carried out on silicagel plates in a Benzene : Diethylether (75:25) solvent system. Vapor phase chromatography (VPC) was performed at 205° C on a MICROTEK 220 chromatograph with ionisation detection. The column was packed with CARBOWAX 20 M 10 % TLC and VPC showed two peaks. The overall yield of the two products was 81 %. These two products were identified by ^{13}C-NMR spectra.

NMR chemical shifts :

* D-arabitol (D_2O) :
  δ = 69.52 ppm (C_3) ; δ = 69.06 ppm (C_2) ;
  δ = 68.82 ppm (C_4) ; δ = 61.58 ppm (C_1) ;
  δ = 61.50 ppm (C_5).
* 2,3-4,5-di-O-isopropylidene D-arabitol :
  δ = 82.00 ppm (C_2) ; δ = 78.63 ppm (C_3) ;
  δ = 77.79 ppm (C_4) ; δ = 68.19 ppm (C_5) ;
  δ = 63.19 ppm (C_1).
* 1,2-4,5-di-O-isopropylidene-D-arabitol :
  δ = 77.65 ppm (C_2) ; δ = 77.02 ppm (C_4) ;
  δ = 73.30 ppm (C_3) ; δ = 68.00 ppm (C_1) ;
  δ = 66.67 ppm (C_5).

2,3-4,5-di-O-ISOPROPYLIDENE-1-O-METHANE SULFONYL-D-ARABITOL (III)

was prepared from the mixture of the two isomers obtained in the previous step by following the method of CEGLA and MANGOLD (23). TLC was carried out on silicagel plates in a Cyclohexane : Diethyl- ether (50:50) solvent system. Two spots were detected (overall yield : 89 %).

The purification was made on a MERCK silica H column with the same solvent system. Two products were obtained, one was the required product : III (checked by NMR) and amounted to 2/3 of the total quantity.

NMR chemical shifts :

* 2,3-4,5-di-O-isopropylidene-1-O-methane sulfonyl-D-arabitol
  δ = 110.50 and 110.80 ppm (C isopropyl.) ;
  δ = 79.32 ppm (C_2) ; δ = 78.15 ppm (C_3) ;
  δ = 77.94 ppm (C_3) ; δ = 69.75 ppm (C_1) ;
  δ = 68.52 ppm (C_4) ; δ = 37.68 ppm (SO_2CH_3) ;
  δ = 27.71 ppm - 27.56 ppm - 27.38 ppm - 28.85 ppm (CH_3 isopropyl.)
* 1,2-4,5-di-O-isopropylidene-3-O-methane sulfonyl-D-arabitol
  δ = 110.32 ppm (C isopropyl.) ; δ = 81.57 ppm (C_3) ;
  δ = 67.03 ppm (C_1) ; δ = 66.72 ppm (C_5) ;
  δ = 39,54 ppm (SO_2CH_3) ; δ = 27.11 ppm - 26.80 ppm - 26.29 ppm - 26.07 ppm (CH_3 isopropyl.)

1-DEOXY-2,3-4,5-di-O-ISOPROPYLIDENE-1-IODO-D-ARABITOL (IV)

was prepared according to BAYLY and TURNER (19). The carbon tetrachloride extracts were filtered and evaporated to dryness. A brown-orange coloured syrup was obtained (yield 113 %).

TLC was carried out on MERCK silicagel plates with chloroform as eluent solvent. The purification was performed on a MERCK silica H column with the same solvent to eliminate the residual mesylate. This iodo compound was stocked at 4° C, in darkness, in chloroform solution, at a concentration of 333 moles/ml.

2-DEOXY-3,4-5,6-di-O-ISOPROPYLIDENE-D-(1-^{14}C) GLUCONONITRILE (V)

In a small pear-shaped flask, 15 µl (5 µmoles) of stock chloroform solution of IV were taken to dryness under reduced pressure. The residue was then dissolved in 200 µl of anhydrous dimethylformamide. In another similar flask, were evaporated together 10 µl (4 µmoles) of a non radioactive sodium cyanide solution in methanol (20 mg/ml) and 10 µl of hydroxyde free sodium (^{14}C)-cyanide solution (33 µCi ; 0.74 µmole).

The solution of (IV) in anhydrous dimethylformamide (DMF) was added onto the dry cyanide. The flask was then stoppered and plunged in an oil bath at 100° C for 2 minutes. The yield of the reaction as a function of time was studied by TLC (silicagel, chloroform). The maximum yield was obtained after 2 minutes (75 - 80 % based on cyanide). A longer period of heating resulted in the formation of another unidentified product with a reduction in the yield of (V).

The DMF solution was immediately stirred with 200 µl of distilled water. This mixture was extracted with 2 ml of diethylether in four 0.5 ml portions. The activity in the ethereal phase was about 70 % of the total. The diethylether extracts were transferred and evaporated in the reduction flask under nitrogen.

NMR chemical shifts :

δ = 118.10 ppm (CN) ;
δ = 111.70 ppm - 111.20 ppm (C isopropyl.) ;
δ = 41.80 ppm (C_2) ; δ = 38.15 ppm (C_4) ;
δ = 37.00 ppm (C_3) ; δ = 69.15 ppm (C_1 + C_5) ;
δ = 28.05 ppm - 27.95 ppm - 26.20 ppm - 23 ppm (CH_3 isopropyl.).

2-DEOXY-D-(1-^{14}C) GLUCOSE (VI)

Catalyst, pressure, temperature and time influences on the reaction yield were studied.

To the previous residue were added 300 µl of HCl 0.5 N and 5 mg of Palladium Black (MERCK art. 807104). The mixture was hydrogenated at atmospheric pressure and 95° C for 10 minutes. It was then filtered through a small column of DOWEX OH^- resin. The eluate was evaporated to dryness.

In preliminary runs, TLC was carried out on cellulose sheets in an Ethylacetate : Pyridine : Water (70:30:20) solvent system. 2-deoxyglucose at the same R_F that of a reference standard was located (brown violet colour) by spraying with an anisidine hydrochloride solution. At this stage, a radioscanning of the TLC sheets showed that the yield of (VI) was to 40-50 % based on nitrile (V) or 30 % based on sodium cyanide. Other products were present : the corresponding amine, (1-amino-1,2-di-deoxy-D-glucitol) a small amount of gluconononitrile and an unidentified impurity.

The purification of (VI) was performed by HPLC with refractive index and radioactivity detectors. An analytical PARTISIL PXS 10/25 PAC WHATMAN column was used with an elution system (1.5 ml/min) Acetonitrile : Water (80:20) adjusted at pH 5 by H_3PO_4. The elution time of (VI) was 5 minutes. The peak corresponding to (VI) was collected and its purity confirmed by TLC and autoradiography (98 %).

NMR chemical shifts :

* 2-deoxy-D- β-glucose (D_2O)
  δ = 93.80 ppm (C_1) ; δ = 78.00 ppm (C_5) ;
  δ = 72.00 ppm (C_3) ; δ = 68.00 ppm (C_4) ;
  δ = 68.91 ppm (C_6) ; δ = 39.20 ppm (C_2).
* 2-deoxy-D- α-glucose (D_2O)
  δ = 91.40 ppm (C_1) ; δ = 71.20 ppm (C_3) ;
  δ = 71.00 ppm (C_5) ; δ = 70.60 ppm (C_4) ;
  δ = 61.00 ppm (C_6) ; δ = 37.00 ppm (C_2).
* 1-amino-1,2-di-deoxy-D-glucitol
  δ = 98.80 ppm (C_3) ; δ = 97,00 ppm (C_2) ;
  δ = 93.95 ppm (C_4) ; δ = 88.99 ppm (C_6) ;
  δ = 63.35 ppm (C_3) ; δ = 56.37 ppm (C_1).

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