SYNTHESIS OF D-XYLOSE, LABELED WITH TRITIUM IN 5 POSITION, AND ITS DERIVATIVES, 1,2,4-ORTHOACETYL-3-O-ACETYL-α-D-XYLOPYRANOSE-5-^{3}H AND 1,5-ANHYDRO-2,3-DI-O-ACETYL-β-D-XYLOFURANOSE-5-^{3}H

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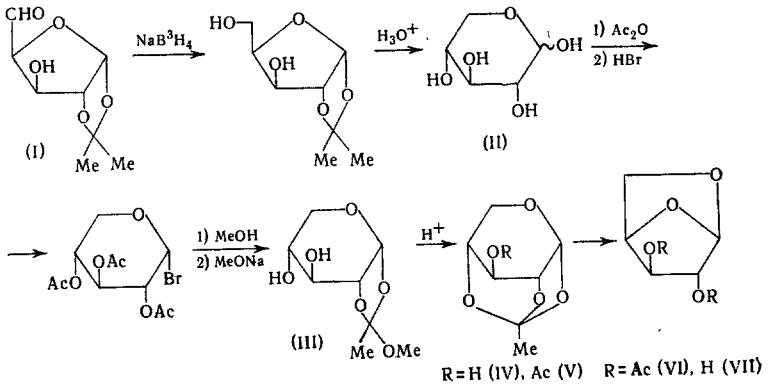
Sugars and their derivatives, labeled with tritium, arouse the interest of investigators due to the convenience of inserting the isotope, its relative low cost, and the small biological hazard of application [1]. In particular, tritium-labeled D-xylose can find broad application in a number of chemical and biochemical studies as a substitute for the expensive and difficultly available radiocarbon-labeled xylose. However, the described synthesis of labeled xylose from dialdose (I) includes the preparatively inconvenient reduction with LiB^3H_4, the availability of which is limited [2]. We developed an alternate synthesis of tritium-labeled D-xylose (II) by the reduction of dialdose (I) with the readily available and highly active commercial NaB^3H_4, which permits running the reduction in water employing the simplest experimental technique. The thus obtained D-xylose-5-^{3}H (specific radioactivity 32 μCi/mole) was then used to synthesize the orthoesters and 1,5-anhydride, which, on the one hand, are needed to study the polymerization of sugar orthoesters (cf. [3]), and on the other hand, are potential intermediates for the synthesis of glycosides and oligosaccharides that contain a labeled xylose moiety



The synthesis of the bicyclic (III) and tricyclic (IV) orthoesters of α-D-xylopyranose was described previously [4], in which connection it was proposed to use molecular sieves for the final cyclization [5], which permitted running the synthesis on a small scale. The indicated methods were transferred to the synthesis of the labeled derivatives, with the difference that all six steps from the labeled D-xylose to the tricyclic orthoester (V) were run without isolating the intermediates except for their chromatographic characterization. In this way orthoester (V) was obtained in 22% yield when based on the xylose, and 21% when based on the radioactivity, and was identified by comparing with the authentic unlabeled specimen.

The isomerization of the tricyclic 1,2,4-orthoesters of α-D-xylopyranose to 1,5-anhydro-β-D-xylofuranose derivatives was discovered and studied in detail on the example of the orthobenzoates [6], whereas for orthoacetate (V) this reaction was only ascertained chromatographically. We studied two variations of the isomerization of orthoester (V): in refluxing chlorobenzene in the presence of collidinium

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perchlorate, and in refluxing nitromethane in the presence of $HgBr_2$. Diacetate (VI) was obtained in both cases, in which connection the yield was substantially higher in the second variation.

The structure of diacetate (VI) was confirmed in the following manner. Its deacetylation leads to the known anhydride (VII). The NMR spectrum of diacetate (VI) contains two three-proton singlets of the acetyl groups and the signals of the ring protons, which in their chemical shifts are very close, while in the values of the SSCC (spin–spin coupling constants) they practically coincide with the signals of the corresponding protons in the NMR spectrum of 1,5-anhydro-2,3-di-O-benzoyl-β-D-xylofuranose [6]. In particular, the SSCC of the protons in the head of the bridge have values that are characteristic for dioxanorbornane systems, and specifically $J_{1,2} = J_{4,5} = 0$, $J_{3,4} = 5$, $J_{4,5'} = 3$ Hz (cf. [6, 7]).

In conclusion, the isomerization of the labeled orthoester (V) in nitromethane gave the labeled anhydride (VI) in 49% yield when based both on compound and on label.

EXPERIMENTAL METHOD

The nitromethane, chlorobenzene, dichloroethane, and Al_2O_3 were prepared as described in [8, 9]. The TLC was run on either silica gel or Al_2O_3 in the systems: $CHCl_3$–Me_2CO, 50:50 (A), 60:40 (B), and 95:5 (C). The GLC was run on an LCM-8MD, Model 5, instrument, using a 1-m steel column packed with 3% PNPPS deposited on Chromatone NL-AW-HMDS, N_2 as the carrier gas, a flame-ionization detector, and a temperature of 135°C. The counting rate was measured on an Isocap-300 liquid scintillation counter, manufactured by Nuclear Chicago, in either a toluene or a dioxane scintillator using toluene-^{3}H of known specific radioactivity as the internal standard. The melting points were determined on a Kofler stand, while the specific rotations were measured on a Perkin–Elmer-141 polarimeter. The NMR spectra were taken on a Varian-DA-60-IL spectrometer in CCl_4 solution relative to TMS. All of the solutions were evaporated in vacuo at 40°.

All of the obtained compounds were chromatographically homogeneous in the indicated solvent systems. All of the labeled compounds were identified by direct comparison with authentic unlabeled specimens. All of the orthoesters were cleaved completely under the conditions of the hydrolytic test for sugar orthoesters [9].

D-Xylose-5-^{3}H (II). With slight modifications, the starting 1,2-O-isopropylidene-α-D-xylo-pentadialdo-1,4-furanose (I) was synthesized as described in [10]. A solution of 2.16 g (9.50 mmoles) of $HIO_4 \cdot 2H_2O$ in 25 ml of water was adjusted to pH 7 by adding solid Na_2CO_3. To the obtained solution at 0° was added in small portions 2.00 g (9.10 mmoles) of 1,2-O-isopropylidene-α-D-glucofuranose in 30 min. After 45 min was added 0.5 ml of ethylene glycol, and the solution was demineralized with anionite IRA-410 (HCO_3^-) and cationite KU-2 H^+). The resins were separated, washed with water, and the filtrates were evaporated to dryness. From the residue we obtained 1.66 g (97%) of dialdose (I), $[\alpha]_D$ –25.3° (C 1.0, equilibrium, water). The TLC was run in system A.

A solution of 94 mg (0.50 mmole) of (I) in 0.5 ml of 0.01 N borate buffer solution (pH 10.0) was added to a fresh portion of commercial NaB^3H_4 (2.4 mg, 0.063 mmole, 100 μCi). The solution was let stand for a day, then 9 mg (0.24 mmole) of $NaBH_4$ was added, and the whole was let stand overnight. Cationite KU-2 (H^+) was then added until weakly acid, the resin was separated, the solution was evaporated to dryness, the residue was treated with 0.3 ml of AcOH and 5 ml of MeOH, and the solution was repeatedly evaporated with MeOH until all of the H_3BO_3 was removed. To the residue was added 230 mg of pure D-xylose, and the mixture was recrystallized from 0.5 ml of MeOH, which contained 1 volume % of 95% AcOH. We obtained 222 mg (73%) of (II), mp 144-146°, $[\alpha]_D$ +19.1° (C 1.0, equilibrium, water), specific activity 32 μCi/mmole (yield based on tritium 47%). Chromatograph on paper FN-11 (butanol–pyridine–water, 6:4:3), run as described in [11], with counting of the radioactivity from the chromatogram revealed that the xylose zone contains 96% of all of the radioactivity.

1,2,4-Orthoacetyl-3-O-acetyl-α-D-xylopyranose-5-^{3}H (V). The acetylation of 56 mg (0.38 mmole) of (II) with 0.63 ml of Ac_2O in 1.26 ml of pyridine was run at 0° for 26 h, then 0.4 ml of MeOH was added, and after 30 min the mixture was diluted with 50 ml of $CHCl_3$, washed in succession with ice water (15 ml), chilled saturated $NaHCO_3$ solution (15 ml), and ice water (2 × 15 ml), and then evaporated to dryness, with removal of the pyridine by azeotropic distillation with heptane. The sirupy residue was treated with 1.375 g (4.33 mmoles) of β-D-xylose tetraacetate, and the mixture was dissolved in 10 ml of a 0° saturated solution of HBr in glacial AcOH and let stand for 3 h. Then it was diluted with 80 ml of $CHCl_3$, washed in succession with ice water (30 ml), chilled saturated $NaHCO_3$ solution (2 × 30 ml), and ice water (2 × 30 ml),

filtered through cotton wadding, and evaporated to dryness. The semicrystalline residue was dried in vacuo, dissolved in a mixture of 7.5 ml of $MeNO_2$, 1 ml of absolute 2,6-lutidine, and 0.2 ml of absolute MeOH, and let stand for 60 h. To the mixture were added 11 ml of Me_2CO, 5 ml of water, and 3 ml of 2 M $AgNO_3$ solution. After 10 min the precipitate was filtered, and the filtrate was diluted with 80 ml of a 1:3 $CHCl_3$–hexane mixture. The organic layer was separated, washed in succession with water (20 ml), saturated NaCl solution (2 × 20 ml), and water (3 × 20 ml), and evaporated to dryness, removing any residual lutidine by repeated distillation with heptane. The residue was dissolved in 10 ml of a 0.01 N MeONa solution in absolute MeOH, and after 2 h the mixture was neutralized with CO_2 and evaporated to dryness. The residue was extracted with ether (4 × 20 ml). Evaporation of the combined extracts gave the sirupy orthoester (III), which was cyclized, as described for the unlabeled analog [5], in 10 ml of dichloroethane in the presence of 4.06 mg of p-toluenesulfonic acid (the reaction was checked by TLC in system B). To the mixture was added 0.1 ml of pyridine, which was then cooled, diluted with 50 ml of $CHCl_3$, washed in succession with ice water (10 ml), chilled saturated $NaHCO_3$ solution (10 ml), and ice water (2 × 10 ml), and evaporated to dryness. The residue was acetylated with 1 ml of Ac_2O in 2.5 ml of absolute pyridine (20°, 24 h), and the reaction mixture was worked up as described above for the acetylation of xylose. The obtained acetate was recrystallized from ethanol to give 226 mg (22% yield) of (V), mp 68°; $[\alpha]_D$ + 63.8° (C 1.0, $CHCl_3$). The TLC was run in system C. The specific radioactivity was 2.4 μCi/mole, and the yield based on tritium was 21%.

1,5-Anhydro-2,3-di-O-acetyl-β-D-xylofuranose and 1,5-Anhydro-2,3-di-O-acetyl-β-D-xylofuranose-5-^{3}H (VI). a) A solution of 1.94 g (9.00 mmoles) of orthoester (V) in 25 ml of chlorobenzene was refluxed for 30 min in a flask, equipped with a reflux condenser and a head, containing 4 Å molecular sieves [5]. Then a solution of 19.9 mg (0.09 mmole) of 2,4,6-collidinium perchlorate, obtained in the same manner as 2,6-lutidinium perchlorate [8], in 5 ml of chlorobenzene, which was dehydrated by azeotropic distillation the same as described in [5], was added. The mixture was refluxed for 4 h until the orthoester had disappeared (checked by the hydrolytic test [9] and TLC in system C), after which 5 ml of pyridine and 500 ml of $CHCl_3$ were added, and the mixture was washed with water (3 × 100 ml) and evaporated until all of the pyridine had been removed. The residue was dissolved in a mixture of 60 ml of Me_2CO and 0.6 ml of 1 N H_2SO_4 solution, let stand for 1 h, neutralized with pyridine, evaporated to ~10 ml, diluted with 500 ml of $CHCl_3$, washed with water (3 × 100 ml), and evaporated. Anhydride (VI) was isolated from the residue by preparative chromatography on Al_2O_3 (15 × 1 cm, elution with benzene). The yield was 250 mg (13%), $[\alpha]_D$ + 9.5° (C 1.0, $CHCl_3$). Found: C 50.07; H 5.70%. $C_9H_{12}O_6$. Calculated: C 50.00; H 5.56%. The NMR spectrum in CCl_4 contains the signals (δ, ppm): 2.03 s (3H, $-COCH_3$); 2.07 s (3H, $-COCH_3$); 3.35 d.d.d. (1H; $J_{5'5} = 7$, $J_{5',4} = 3$, $J_{5'3} = 1.25$ Hz, $H^{5'}$); 3.81 d (1H, $J_{5,5'} = 7$ Hz, H^5); 4.55 d (1H, $J_{2,3} = 1.5$ Hz, H^2); 4.69 d.d.d. (1H, $J_{3,4} = 5$, $J_{3,2} = 1.5$, $J_{3,5'} = 1.25$ Hz, H^3); 4.89 d.d. (1H, $J_{4,3} = 5$, $J_{4,5'} = 3$ Hz, H^4); 5.28 d (1H, $J_{1,5} = 0.75$ Hz, H^1). The spin relation of all of the vicinal pairs of protons and the further couplings of H^3 with $H^{5'}$, and of H^1 with H^5, were confirmed by the double resonance data.

Diacetate (VI) (128 mg, 0.593 mmole) was deacetylated with 0.01 N MeONa in absolute MeOH solution, after which the solution was neutralized with KU-2 ($C_5H_5NH^+$), the resin was filtered, washed with MeOH, and the combined filtrates were evaporated to dryness. The residue crystallizes from the sirup after evaporation of the acetone solution to dryness. The yield of (VII) was 66 mg (84%), mp 95-97°, $[\alpha]_D$ −12.0° (C 1.0, EtOH). Based on the IR spectrum and the absence of a mixed melting point depression, the compound is identical with an authentic specimen [6].

b) A solution of 432 mg (2.00 mmoles) of orthoester (V) in 15 ml of $MeNO_2$ was treated with 144 mg (0.40 mmole) of $HgBr_2$, as described for the chlorobenzene variation (checked by TLC and GLC), after which the mixture was diluted up to 50 ml with Me_2CO, 0.5 ml of 1 N H_2SO_4 solution was added, and then it was worked up as described above. The yield of anhydride (VI) was 250 mg (58%). Based on the TLC (system C) and GLC, the compound is identical with the specimen from the preceding experiment.

c) The labeled anhydride (VI) was obtained from 108 mg (0.50 mmole) of orthoester (V) (labeled (V), the synthesis of which was described above, diluted in half with the unlabeled specimen), as described in variation b). After distillation in a high vacuum (~10^{-5} mm, ~100°), the yield of (VI) was 53 mg (49%), the specific activity was 1.2 μCi/mmole, and the yield based on tritium was 49%.

CONCLUSIONS

1. Starting with 1,2-O-isopropylidene-α-D-xylo-pentadialdo-1,4-furanose and NaB^3H_4 we synthesized D-xylose-5-^{3}H with a high specific radioactivity, and from it 1,2,4-orthoacetyl-3-O-acetyl-α-D-xylopyranose-5-^{3}H.

2. We synthesized and characterized 1,5-anhydro-2,3-di-O-acetyl-β-D-xylofuranose and its isotopic analog, labeled with tritium in the 5 position.

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