Carbohydrate Research, 28 (1973) 158–164

Note

9-α-D-Xylofuranosyladenine. Acetolysis of 3,5-di-*O*-acetyl-1,2-*O*-isopropylidene-α-D-xylofuranose

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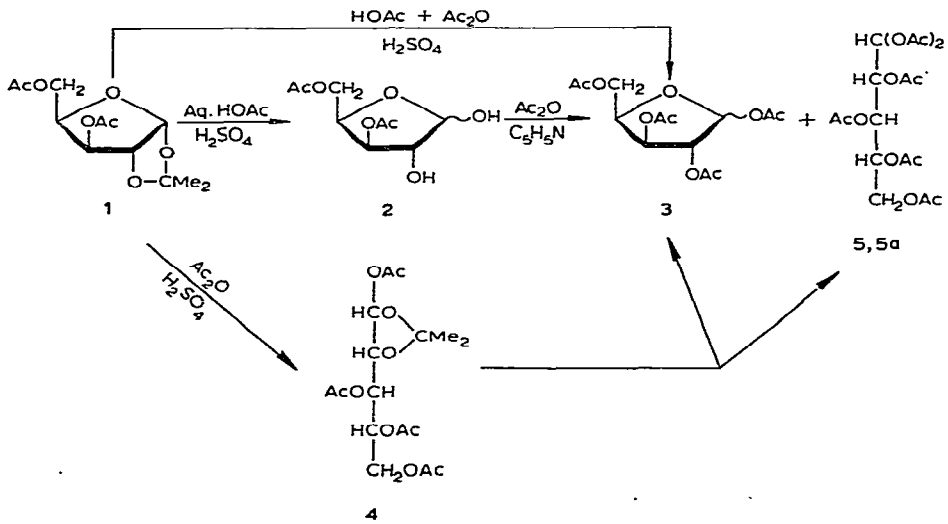
(Received December 26th, 1972; accepted with revisions February 3rd, 1973)

Condensing 2,3,5-tri-*O*-acetyl-D-xylofuranosyl chloride with chloromercuri-6-benzamidopurine[1,2] gave 9-α and β-D-xylofuranosyladenine and an additional product, hexa-*O*-acetyl-*aldehydo*-D-xylose aldehydrol[3] (**5**), that had been a contaminant in the precursor tetra-*O*-acetyl-D-xylofuranose (**3**) obtained by acetolysis[6] of 3,5-di-*O*-acetyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**1**) with acetic acid–acetic anhydride–sulfuric acid.



DISCUSSION

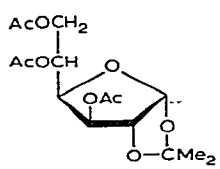
Acetolysis in acetic acid–acetic anhydride with sulfuric acid catalysis has commonly been used to prepare furanosyl acetates from furanoses and furanosides[5–8]. This convenient reaction can, however, give by-products not readily removed from non-crystalline products.

Acetolysis of **1** overnight in acetic anhydride and a sulfuric acid catalyst gave exclusively the acyclic hexaacetate[3] **5**. Fission of the furanose ring was quite rapid, as acetolysis for a short time (5–10 min) gave (t.l.c.) a quantitative yield of the acyclic tetra-*O*-acetyl-1,2-*O*-isopropylidene-*aldehydo*-D-xylose (**4**). Syrupy compound **4** exhibited a doublet at τ 3.8 in its n.m.r. spectrum, indicating[3] that an acetoxyl group was present at C-1. Irradiation of **4** at τ 5.5 collapsed the H-1 doublet to a singlet, showing that the H-2 signal of **4** remained in the region of τ 5.5; the H-4 signal had also shifted downfield because of acetylation at O-4.

1,2-*O*-Isopropylidene-α-D-xylofuranose (**1**) and its acetolysis products **3**, **4**, and **5** were treated overnight with 10:1.2:0.7 acetic acid–acetic anhydride–sulfuric acid, a mixture commonly used for the acetolysis of furanoses. The reactions were monitored by t.l.c. and the products obtained examined by n.m.r. and by gas chromatography.

Compound **1** gave a mixture of **3** and **5** in a ratio of 8:1, together with 2% of another product presumed to be the tetraacetoxypyranose; compound **3** gave a mixture of **3** and **5** in a ratio of 6:1. Compound **4** gave a complex mixture consisting of **3** (27%), **5** (36%), 4% of the pyranose fraction, and 32% of another hexaacetate (**5a**) that differed from **5** in t.l.c., n.m.r. and g.l.c. Compound **5** was recovered unchanged. The results show that the acetolysis mixture can cause ring fission and that subsequent ring-closure can occur.

There has been controversy over the structure of the product obtained from 1,2-*O*-isopropylidene-α-D-glucofuranose or its 3,5,6-triacetate (**6**) by acetolysis in acetic anhydride with sulfuric acid or zinc chloride as catalyst. Brigl *et al.*[9] favored the acyclic structure **8**, whereas Schlubach *et al.*[10] opted for structure **7**, arguing mainly that fission of the ring would not be succeeded by ring closure. N.m.r. spectral analysis of the acetolysis product (acetic acid–sulfuric acid) showed, as with the acyclic xylose derivative **4**, that H-2 of the glucose derivative was not shifted, whereas the H-4 signal had shifted downfield because of acetylation. The doublet observed at τ 3.8 for H-1 afforded additional evidence that the acetolysis product is the *aldehydo*-glucose derivative **8**.



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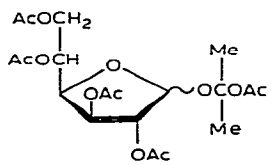

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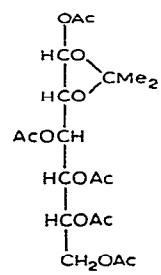

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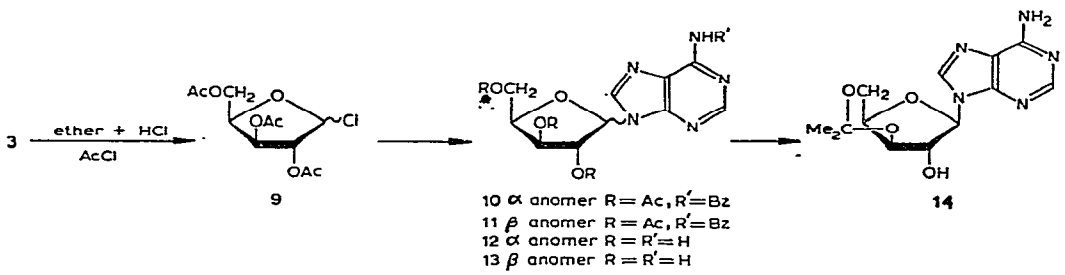
To prepare pure D-xylofuranose tetraacetate (**3**), it was found best to deacetonate compound **1** and then acetylate the resulting 3,5-di-*O*-acetyl-D-xylofuranose (**2**), with or without isolation of the intermediate **2**. The tetraacetate **3** was converted by hy-

drogen chloride in ethereal solution containing acetyl chloride[7] into the furanosyl chloride **9**, and the latter was condensed with 6-benzamido-9-chloromercuripurine in boiling xylene for 2 h to give the acylated nucleosides **10** and **11** in a ratio of 1:3. A reaction time of 15 min led to equal amounts of the anomeric nucleosides. The acylated nucleosides were separated and purified by a combination of crystallization and column chromatography, and the α anomer **10** was isolated crystalline. Deacylation with sodium methoxide or by refluxing in methanol containing sodium hydrogen carbonate gave the free 9-α- and β-D-xylofuranosyladenines **12** and **13**; the α anomer **12** was crystalline. For further identification, the β anomer **13** was converted into its known[1,2] 3,5-isopropylidene acetal **14**.



EXPERIMENTAL

General methods. — Solutions were evaporated under diminished pressure at 40–45°. Melting points were determined with a Thomas–Hoover "Unimelt" apparatus and are not corrected. Optical rotations were taken on a Perkin–Elmer 141 polarimeter. N.m.r. spectra were run in chloroform-*d* unless otherwise specified, with Varian T-60 and Jeol Model C60H instruments. Spin-decoupling experiments were performed by John Messina of these laboratories. Column chromatography was conducted with silica gel (No. 7734, E. Merck, Darmstadt, Germany) and t.l.c. with silica gel GF plates (Analtech, Inc., Wilmington, Delaware). Gas chromatography was conducted by Dr. E. White of these laboratories. Samples were analyzed on a Barber–Colman 5000 instrument equipped with flame-ionization detectors. The column was glass, 2 m × 4 mm (i.d.), packed with 3% OV-225 on 80–100 mesh Chromosorb W-High Performance (Ohio Valley Specialty Chemical Co., Marietta, Ohio). The flow rate of helium carrier gas was 60 ml/min and the column was programmed at a rate of 1°/min from 140° to 200°. The injection port was kept at 210° and the detector at 300°.

1,2,3,5-Tetra-O-*acetyl*-D-*xylofuranose* (**3**). — Sulfuric acid (1 ml) was added to a solution of 3,5-di-*O*-acetyl-1,2-*O*-isopropylidene-α-D-xylofuranose[4] (**1**, 27 g) in 85% aqueous acetic acid (100 ml) and the stirred mixture was heated for 1 to 2 h at 50°, until t.l.c. showed complete conversion of **1**. The mixture was concentrated *in vacuo* to one-half its volume, pyridine (20 ml) was added, and acetic anhydride (125 ml) was added dropwise with stirring, maintaining the temperature at 50–55°. The mixture was

heated for an additional h, and after concentration *in vacuo* the product was isolated by ether extraction to afford **3** (30 g) as a pale yellow syrup. A sample was purified on a column of 10 parts of silica gel, eluted with 1:1 cyclohexane–ethyl acetate, to give pure **3** (95% recovery), $[\alpha]_D^{20}$ +37° (*c* 1, chloroform)[8]. Its n.m.r. spectrum indicated equal parts of the α anomer (doublet at τ 3.55, $J_{1,2}$ 5 Hz, H-1) and the β anomer (singlet at τ 3.85). The gas chromatogram showed twin peaks of equal intensity, together with a small amount (<1%) of a fraction giving two peaks having slightly longer retention times (α and β pyranose anomers).

Anal. Calc. for $C_{13}H_{18}O_9$: C, 49.06; H, 5.70. Found: C, 48.86; H, 5.65.

Alternatively, after deacetonation of **1** and concentration of the solution, the resultant 3,5-di-*O*-acetyl-D-xylofuranose (**2**) was extracted with chloroform and acetylated with acetic anhydride–pyridine in the usual manner.

*Acetolysis of 3,5-di-*O*-acetyl-1,2-*O*-isopropylidene-α-*D*-xylofuranose* (**1**). — *With acetic acid–acetic anhydride–sulfuric acid*[6]. To a cooled mixture of acetic acid (200 ml), acetic anhydride (25 ml), and sulfuric acid (15 ml) was added 21 g of compound **1**. The solution was kept overnight, poured into ice and water, and stirred for 30 min. Extraction with ether followed by washing the extract with water and sodium hydrogen carbonate afforded a pale yellow syrup (25 g). Although the product migrated essentially as a single spot by t.l.c. (1:1 cyclohexane–ethyl acetate), the presence in its n.m.r. spectrum of a doublet at τ 3.2, a doublet at τ 3.55, and a singlet at τ 3.85 showed it to be a mixture; it was resolved by repeated column chromatography on silica gel (2:1 cyclohexane–ethyl acetate) to afford the tetraacetate **3** (R_F 0.385) and the acyclic hexaacetate **5** (R_F 0.375) in a ratio of 8.5:1.

With acetic anhydride–sulfuric acid. A. Compound **1** (780 mg) in a mixture of acetic anhydride (10 ml) and sulfuric acid (0.7 ml) was kept overnight at room temperature. Work up as already described yielded hexa-*O*-acetyl-*aldehydo*-D-xylose aldehydrol (**5**) (800 mg) as a pale yellow syrup, $[\alpha]_D^{20}$ +5.3° (*c* 1, chloroform); n.m.r. τ 3.2d, $J_{1,2}$ 5 Hz (H-1). Gas chromatography showed only one peak.

Anal. Calc. for $C_{17}H_{24}O_{12}$: C, 48.57; H, 5.75. Found: 48.12; H, 5.72.

B. To a stirred mixture of acetic anhydride (10 ml) and sulfuric acid (0.7 ml) at 15° was added **1** (1 g). T.l.c. monitoring showed that decomposition of **1** was complete after several min. After 5 min the solution was poured into ice and water containing sodium acetate. Extraction with ether afforded a product formulated as 1,3,4,5-tetra-*O*-acetyl-1,2-*O*-isopropylidene-*aldehydo*-D-xylose (**4**, 1.05 g) as a pale-yellow syrup; $[\alpha]_D^{25}$ +40.4° (*c* 1, chloroform); n.m.r. τ 3.85d (one proton, $J_{1,2}$ 2 Hz, H-1), 4.6–4.8 m (two protons, H-3, H-4), and τ 5.5–6.0m (three protons, H-2, H-5); irradiation at τ 5.55 collapsed the H-1 doublet to a singlet. T.l.c., R_F 0.45 (2:1 cyclohexane–ethyl acetate); the gas chromatogram showed a single main peak, with traces of **3** and **5** present.

Anal. Calc. for $C_{16}H_{24}O_{10}$: C, 51.06; H, 6.43. Found: C, 50.82; H, 6.13.

In a similar reaction performed for 15 min at room temperature, the product consisted of **4** (75%), **3** (21%), and hexaacetate **5** (3%).

General conditions for acetolysis of compounds **1**, **3**, **4** *and* **5**. — Acetolysis

reactions in 10 ml of a mixture of acetic acid (100 ml), acetic anhydride (12 ml), and sulfuric acid (7 ml) were run for 30 h at room temperature with 660 mg of each compound. After conventional isolation, the products were examined by t.l.c., n.m.r., and gas chromatography. Percentages of the products obtained were determined from the n.m.r. spectra and by gas chromatography; the estimated percentage of the pyranose fraction present was corrected by subtracting the amount of any starting material present. T.l.c. plates were developed with 2:1 cyclohexane–ethyl acetate.

Acetolysis of compound **1**. — T.l.c. showed the product to be mostly **3** (R_F 0.385) together with some **5** (R_F 0.375). The n.m.r. spectrum exhibited a doublet at τ 3.2 ($J_{1,2}$ 5 Hz) for the hexaacetate **5**, a doublet at τ 3.55 ($J_{1,2}$ 5 Hz) for the α anomer of **3**, and a singlet at τ 3.85 for the β anomer of **3**; the integrated spectrum indicated that these products were present in a ratio of 1:4.25:4.25. The gas chromatogram showed twin peaks for acetylated pyranoses (2%), twin peaks for **3** (87%), and a single peak for **5** (11%).

Acetolysis of compound **3**. — By t.l.c. and n.m.r. spectroscopy, the product was similar to that from compound **1**. Gas chromatography indicated the presence of **3** (84%), **5** (15%), and a trace only of the pyranoid fraction.

Acetolysis of compound **4**. — Although the product was similar by t.l.c. and n.m.r. to those just described, the n.m.r. spectrum showed an additional doublet at τ 3.15 ($J_{1,2}$ 1.5 Hz) suggesting the presence of an acyclic hexaacetate **5a** differing in structure from **5**. By gas chromatography the mixture contained a pyranose fraction (4%), compound **3** (27%) as twin peaks, compound **5a** (32%) as a single peak, and **5** (36%) as a single peak. Compounds **3** and **5a** were not separable by column chromatography, but mixed fractions were collected; each contained about 30% of **5a** by n.m.r. but migrated as a single spot by t.l.c. The integrated spectra of these fractions confirmed that they were mixtures of tetra- and hexa-acetates. Hexaacetate **5a** may have formed in the acetolysis mixture by an epimerization reaction[11] of **4**.

Attempted acetolysis of compound **5**. — Hexaacetate **5** was recovered unchanged.

*Acetolysis of 3,5,6-tri-*O*-acetyl-1,2-*O*-isopropylidene-α-*D*-glucofuranose* (**6**)[10]. — To a solution of the acetal **6** (2 g) in acetic anhydride (5 ml) was added a mixture of acetic anhydride (5 ml) and sulfuric acid (0.5 ml). After 15 min at room temperature the solution was poured into ice and water containing sodium acetate. Work up by ether extraction and crystallization from methanol afforded a penta-*O*-acetyl-1,2-*O*-isopropylidene-*aldehydo*-D-glucose (**8**), m.p. 136°; n.m.r. τ 3.8 (doublet, $J_{1,2}$ 2 Hz (H-1). Irradiation near τ 5.6 collapsed the H-1 doublet to a singlet showing that the H-2 signal had not shifted, and the shift of H-1 from τ 4.15 to τ 3.8 further substantiates the assigned structure **8**.

*2,3,5-Tri-*O*-acetyl-*D*-xylofuranosyl chloride* (**9**). — A solution of the tetraacetate **3** (8 g) in 150 ml of ether containing acetyl chloride (3 ml) was saturated at 10–15° with hydrogen chloride gas[7], and hydrogen chloride was slowly bubbled through the solution for an additional h. The solution was evaporated to a syrup *in vacuo* at 40°, 30 ml of benzene was added, and the evaporation was repeated. Conversion into the chloride, as judged by n.m.r. spectroscopy, was 93–95%. After a second similar

treatment with hydrogen chloride, no tetraacetate could be detected in the spectrum. The ratio of α anomer to β anomer varied from 1:1 to 1:1.5 in several preparations. The syrupy **9** was used directly in the next step.

*9-(2,3,5-Tri-*O*-acetyl-α- and β-*D*-xylofuranosyl)-6-benzamidopurines* (**10** *and* **11**). — Compound **9** (prepared from **3**, 8 g) in dry xylene (25 ml) was added to a stirred mixture of boiling xylene (200 ml), Celite (8.2 g), and 6-benzamido-9-chloromercuripurine (8.85 g) that had been previously dried azeotropically. Slow distillation of xylene was conducted for 2 h. The hot mixture was filtered and the solids were thoroughly washed with chloroform. The filtrate was concentrated *in vacuo* to a solid residue that was dissolved, with the aid of methanol, in the chloroform wash-solution. After washing twice with 30% potassium iodide solution and several times with water, removal of the solvent afforded 10.9 g of a colored, amorphous product that was purified by column chromatography on silica gel (110 g). The less polar fractions were eluted with 2:1 benzene–ethyl acetate and the acylated nucleosides (8.4 g) with 8:1 benzene–methanol. The chromatography was repeated on 170 g of silica gel to purify and to separate the anomers further. Elution with 1:1 benzene–ethyl acetate removed less-polar material, and elution with ethyl acetate removed the α nucleoside **10**, which readily crystallized from ethyl acetate. The β nucleoside **11** was eluted by 10:1 benzene–methanol. By crystallizing **10** and rechromatographing the mother liquors there was obtained 6.75 g of **10** and **11** in a ratio of 1:3. Repeated chromatography gave some more-polar fractions (t.l.c.), probably formed by partial deacetylation on the column when methanol was used.

In a similar preparation but with a heating time of 0.5 h after the addition of the chloride **9**, the ratio of **10** to **11** obtained was 2.5:4.

The acylated nucleosides (16 g) were obtained in a ratio of 1:1 from **9** prepared from 19.1 g of the tetraacetate **3** when the heating time was decreased to 15 min.

Acylated α-nucleoside **10**. — Compound **10** crystallized from ethyl acetate; m.p. 207–209°, $[\alpha]_D^{25}$ −35.8° (*c* 0.98, chloroform); t.l.c. R_F 0.47 (ethyl acetate); n.m.r., H-1′ was buried under the complex multiplets of H-2′ and H-3′ in the region of τ 4.2.

Anal. Calc. for $C_{23}H_{23}N_5O_8$: C, 55.53; H, 4.66; N, 14.08. Found: C, 55.43; H, 4.74; N, 13.73.

Acylated β-nucleoside **11**. — Compound **11** was obtained as an amorphous product; n.m.r. τ 3.72 d ($J_{1,2}$ 2 Hz); t.l.c., R_F 0.40 (ethyl acetate) and a more-polar fraction was present.

*9-α-*D*-Xylofuranosyladenine* (**12**). — Methanol (150 ml) was boiled with sodium hydrogen carbonate (1.5 g) and the warm mixture was filtered. The acylated nucleoside **10** (6 g) was added to the filtrate and the solution was stirred and distilled during a 0.5-h period to remove methanol (50 ml). During this time the product crystallized from the hot solution. The mixture was cooled and filtered to yield the free α-nucleoside **12** (3.1 g), m.p. 305–307°. Recrystallization from 80% aqeuous ethanol raised the m.p. to 308°, $[\alpha]_D^{25}$ −20.4° (*c* 0.46, 5% aqueous trifluoroacetic acid); t.l.c. (3:2 benzene–methanol) R_F 0.46; n.m.r., D_2O (trifluoroacetate) τ 4.25 d, $J_{1,2}$ 9 Hz (H-1′).

Anal. Calc. for $C_{10}H_{13}N_5O_4$: C, 44.94; H, 4.90; N, 26.20. Found: C, 44.83; H, 4.92; N, 26.18.

9-β-D-Xylofuranosyladenine (**13**). — Compound **11** (6 g) was refluxed for 15 min in methanol (90 ml) containing sodium methoxide (600 mg Na). The solid residue obtained after removal of the solvent was repeatedly extracted with hot ethyl acetate. The combined extracts were concentrated to a low volume, diluted with hexane to turbidity, kept for 15 min, and filtered through a bed of Celite. This operation was repeated three times. Final concentration, and removal of the solvent, yielded the β-nucleoside **13** (3.3 g); m.p. 192–195°; $[\alpha]_D^{25}$ −32.1° (*c* 0.5, water); t.l.c., R_F 0.34 (3:2 benzene–methanol); n.m.r. (D_2O) τ 4.27 d, $J_{1',2'}$ 3 Hz (H-1'). Analyses and the n.m.r. spectrum indicated solvation with 0.5 mole of methanol.

A sample of **13** (200 mg) treated for 40 h in acetone (10 ml) with *p*-toluenesulfonic acid (400 mg) gave, upon isolation[1,2], 180 mg of 9-(3,5-*O*-isopropylidene-β-D-xylofuranosyl)adenine (**14**), m.p. 206–208°.

REFERENCES

1 W. W. Zorbach and R. S. Tipson (eds.), *Synthetic Procedures in Nucleic Acid Chemistry*, Interscience Publishers, New York, N.Y., 1968 Vol. 1, p. 139.
2 W. W. Lee, A. P. Martinez, G. L. Tong, and L. Goodman, *Chem. Ind.* (London), (1963) 2007.
3 F. W. Lichtenthaler, J. Breunig, and W. Fisher, *Tetrahedron Lett.*, (1971) 2825.
4 P. A. Levene and A. L. Raymond, *J. Biol. Chem.*, 102 (1933) 317.
5 E. J. Reist, R. R. Spencer, and B. R. Baker, *J. Org. Chem.*, 23 (1958) 1753.
6 E. J. Reist and L. Goodman, *Biochemistry*, 3 (1964) 15.
7 A. Rosenthal and L. Nguyen, *J. Org. Chem.*, 34 (1969) 1029.
8 D. H. Murray and J. Prokop, *J. Pharm. Sci.*, 54 (1965) 1468.
9 P. Brigl and W. Zerrweck, *Ber.*, 66 (1933) 936.
10 H. H. Schlubach, W. Rauchenberger, and A. Schultze, *Ber.*, 66 (1933) 1248.
11 S. Sowa, *Can. J. Chem.*, 50 (1972) 1092.